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FACTORS INFLUENCING THE EXCRETION OF UREA

I. Rate of Urine Excretion and Caffeine

HELEN BOURQUIN

From the Department of Physiology of the University of South Dakota Medical School

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The excretion of urea has been subjected to sufficient investigation to demonstrate that, aside from the functional capacity of the kidneys, the concentration of blood urea is a controlling factor, the ratio $\frac{\text{Urea excretion rate}}{\text{Blood urea concentration}}$ tending to be a constant (1), (3), (4).

The third determining factor according to Ambard (4) is urine urea concentration. Austin, Stillman and Van Slyke (3) present evidence to show that the latter has no influence on rate of urea elimination but that the rate increases "approximately (a) in simple direct proportion to the blood urea concentration, and (b) in proportion to the square root of the rate of volume output of urine per unit body weight as long as the volume rate remains within ordinary limits." The results of Marshall and Crane (5) bear this out in that they find that coincident with the increased volume output of the denervated kidney there is slight but constant increase in rate of urea elimination. In fact that is rather generally conceded to be a characteristic of diuresis.

Addis and Drury (1), (2) on the other hand find that the volume output of urine per se bears no relation to rate of urea excretion but that many minor factors such as ingestion of meals and of physiological saline do influence it.

This investigation was begun before the publication of the last mentioned papers of Addis and Drury with a view to determining whether diuretics believed to act directly on renal epithelium influence the ratio $\frac{\text{Urea excretion rate}}{\text{Blood urine concentration.}}$ The data, of course, bear on both questions.

METHODS. Dogs have been used throughout as the experimental animal. To make the conditions of the experiment as uniform as possible the following procedure was followed.

The animal was deprived of food for twelve hours, water being allowed freely. As a preliminary to the experiment it was placed under ether anesthesia; a tracheal cannula was inserted for administering ether; the right carotid artery was cannulated for blood pressure determination; short cannulae were inserted into the ureters about 3 inches from the pelvis of each kidney and the kidneys were denervated.

It was desired to study the denervated kidney in order to eliminate, in so far as possible, variations in function through changes in blood supply due to vasomotor changes in the kidney itself. That vasoconstriction could and doubtless did occur even under these conditions is shown by the recent work of Stoll and Carlson (7), but certainly such change was minimized.

In denervating the kidney the dorsal approach was used so that the peritoneal cavity need not be opened, and the nerves along the renal artery and vein were severed, the surrounding tissue being also cut as a precautionary measure. Subsequent autopsy verified the success of the operation. The incision made to approach the kidney was extended so that the ureters could be cannulated, the opening then being closed by hemostats. Usually the entire operation consumed an hour or less.

As soon as possible after the insertion of the tracheal cannula 0.3 gram of sodium veronal per kilo in about 200 cc. of water was given by stomach tube, so that shortly after the completion of the operation ether could be dispensed with. In a few cases only was it necessary to use light ether throughout the experiment.

Usually urine was flowing freely from the cannula at the time of its insertion but collection of urine was not begun until 45 minutes or more after the operation was completed. By that time the excretion of both water and urea had reached a uniform rate. In about 50 per cent of the experiments one kidney or the other failed to function sooner or later, or began to do so so gradually that it could not be used. In the first instance kinking of the ureter could always be demonstrated. In the second case a spasm of vasoconstriction (7) was probably responsible.

Urine was collected every fifteen or thirty minutes, certified graduates being used for this purpose throughout most of the work. Urea determinations were made by the urease method with direct Nesslerization using essentially the technic described by Folin and Youngburg (8). Duplicate determinations were made in all cases. Though the excretion of ammonia was found to run a course more or less different from that of the urea in the experiments in which it was determined, it represented such a small per cent of the total urea plus ammonia nitrogen estimated

by this method as not to influence the results perceptibly. For that reason it has not been determined in many of these experiments, the so-called urea nitrogen being urea and ammonia nitrogen unless otherwise specified.

Blood samples were collected from the femoral artery in the middle of each period of urine collection and blood urea determined by the urease method of Folin and Wu (9).

In later experiments freezing point determinations were also made.

All experiments were performed in a very warm room and at the completion of the experiment the animal was still in excellent condition in 15 out of the 17 experiments from which data are taken for this paper. Not only was blood pressure good and the heart beat vigorous but the medullary centers responded to asphyxia in the normal way. That the kidneys were still in good condition is demonstrated by the fact that phenolsulphonephthalein produced slight diuresis and was eliminated at a rate normal to dogs (10).

The experiments fall roughly into two groups. In the first group varying amounts of urea were injected intravenously following a control period of from an hour to an hour and a half. In the second group after a similar control period 1 cc. per kilo of a 0.5 per cent solution of caffeine in 0.9 per cent saline was injected intravenously, a second injection about three-fourths as large being made one to two hours later. In this group the control periods were of three types. *a*, In about one-third of all cases an injection of 1 cc. of 0.9 per cent saline per kilo immediately preceded it; *b*, in over half the cases no injection preceded the administration of caffeine, water diuresis being maintained by giving water by stomach tube at frequent intervals throughout the experiment; *c*, in two instances 1 cc. of 20 per cent saline per kilo was injected in which cases the caffeine was injected in a similar solution.

RESULTS AND DISCUSSION. Results from experiments on 17 animals are included in this series. In experiments in which both kidneys functioned, the results obtained correspond very closely, with one exception to be mentioned later.

Relation between rate of volume output and the $\frac{D}{B}$ ratio. Examination of the results (table 1) shows clearly that there is no direct relation between rate of urine output as expressed in terms of the ratio $\frac{\text{Urea excretion rate}}{\text{Blood urea concentration}}$, $\frac{D}{B}$ ratio, and rate of elimination of water, for neither is increase in rate of volume output accompanied by a higher $\frac{D}{B}$ ratio with any degree of constancy, nor does the ratio always decrease with the lowered rate of volume output. In other words, under experimental

conditions as nearly constant as it seemed possible to make them, it has been found that stimuli influencing rate of water excretion in either a plus or minus direction may or may not alter urea excretion and vice versa. Addis and Drury (1), (2) have drawn essentially the same conclusions from similar results and Adolph (13) presents good evidence that urea excretion is relatively independent of rate of water elimination.

TABLE 1

A summary of results to show the relation between rate of volume output and $\frac{D}{B}$ *ratio*

Protocols typical of those from which this summary is made are included in table 2. Where both kidneys functioned the results were alike except in the case cited below.

	INJECTION FOLLOWING THE CONTROL PERIOD	CHANGES IN RATE OF URINE VOLUME SECRETION FOLLOWING THE INJECTION	CHANGES IN $\frac{D}{B}$ RATIO FOLLOWING THE INJECTION	NUMBER OF EXPERIMENTS
1	0.3 gram of urea per kilo	Temporary diuresis	None	3
2	1.0 gram of urea per kilo	Temporary diuresis	Changes paralleling changes in urine volume	1
3	5.0 grams of urea per kilo	Diuresis with marked volume fluctuations	Constant after an initial increase	1
4	5 mgm. of caffeine per kilo	None	Marked increase	2
5	5 mgm. of caffeine per kilo	Temporary diuresis	Marked increase bearing no relation to urine volume changes	3
6	5 mgm. of caffeine per kilo	Diuresis	Changes paralleling changes in urine volume	1
7	5 mgm. of caffeine†	Diuresis	Slight increase	1
8	5 mgm. of caffeine†	Diuresis	Definite temporary depression	1*
9	5 mgm. of caffeine per kilo	Steady decrease	Similar but more rapid decrease	5

* The left kidney of this experiment responded by diuresis and an increase in the $\frac{D}{B}$ ratio.

† Per kilo.

The fact that conditions which alter the $\frac{D}{B}$ ratio often do alter the output of water in the same direction may explain the results leading Austin, Stillman and Van Slyke (3) to the conclusion that there is a causative relation between the two. Just what the factors are which would produce such a constant parallel effect on water and urea excretion as is

indicated in their results needs further investigation. Theoretically desiccation should be one such condition, but an examination of the high concentration of urine urea, which frequently occurs, indicates that this desiccation would need to be very great. It seems quite certain that it was not the determining factor in the results obtained by the latter investigators.

The relation between caffeine and the $\frac{D}{B}$ ratio. Caffeine definitely raised the $\frac{D}{B}$ ratio in 6 experiments (table 2), produced a slight increase in a seventh, and definitely depressed it in six (table 1). A striking characteristic of the urines in which the $\frac{D}{B}$ ratio was increased was a similar increase in ammonia output and an unchanged or relatively slight lowering of the depression of freezing point values even in the presence of diuresis, both of which indicate an increased activity on the part of the kidney.

These changes in $\frac{D}{B}$ ratio occur irrespective of the presence or absence of diuresis (table 1) or of the nature of the control period. For instance in 2 of the 6 experiments accompanied by an augmented $\frac{D}{B}$ ratio injections of 20 per cent sodium choride solutions were made, in one the control was 1 cc. per kilo of a 0.9 per cent saline solution, in 3 no preliminary injection was made but water was given by stomach tube throughout the experiment. Clearly the changes in the $\frac{D}{B}$ ratio are due to the action of caffeine per se on the rate of urea excretion.

This action may be produced by vasomotor changes in the kidney, by direct action of the caffeine on the renal epithelium or capillaries or both, or by some undetermined blood change.

Underhill (11) finds that in dogs water diuresis is uniformily inhibited over varying periods of time by injections of caffeine or the other related purine derivatives, his dosage being 2 or 3 times as large as that used in these experiments. He gives evidence that this is due to the vasoconstrictor action of caffeine. However the peripheral action of caffeine is known to be vasodilator, the central action to be vasoconstrictor (12). Inasmuch as these kidneys are denervated both influences should be eliminated. At most a greater degree of vasodilatation should occur. These experiments give no data concerning the vasomotor changes actually occurring but they do give evidence that vasomotor changes would not account for the results in the case of the augmentation effects. These effects are entirely different from those produced by Marshall and Crane (5), (6) in their study of the effects of altering the blood supply to a de-

TABLE 2

Experiments demonstrating the augmentation of rate of urea excretion produced by caffeine

EXPERIMENT		PROCEDURE	BLOOD PRESSURE IN MM. OF Hg	BLOOD UREA NITROGEN, GRAMS PER LITER	URINE UREA NITROGEN, GRAMS PER LITER	GRAMS OF UREA NITROGEN IN 24 HOURS	RATIO $\frac{\text{UREA NITROGEN PER 24 HOURS}}{\text{BLOOD UREA NITROGEN}}$	LITERS OF URINE PER 24 HOURS	DEPRESSION OF FREEZING POINT
December 6, mongrel; 16 kilos; left kidney. Right kidney gave same results. Water by stomach tube throughout experiment	9:17	Operation completed							
	10:13	Urine collection begun							
	10:45	Urine collected	128	0.214	16.00	2.57	12.9	0.168	
	11:15	Urine collected	128		17.4	3.75	18.39	0.220	1.495
	11:57	Urine collected	114	0.208	16.1	4.29	20.05	0.264	1.51
	12:00	Caffeine injected; new urine period begun							
	12:16	Urine collected		0.198	20.0	9.38	47.30	0.480	1.735
	12:35	Urine collected	116	0.198	20.0	7.49	37.72	0.384	
	1:05	Urine collected		0.208	15.5	5.81	28.50	0.384	2.04
	1:35	Urine collected			16.83	6.102	29.89	0.377	1.96
	2:03	Caffeine injected							
	2:05	Urine collected		0.207	18.86	6.52	32.00	0.355	1.93
	2:20	Urine collected		0.211	16.65	6.57	32.2	0.408	
	2:35	Urine collected		0.19	16.00	5.77	30.04	0.377	1.98
	3:05	Urine collected	106	0.210	17.10	4.94	23.16	0.302	
	3:35	Urine collected			14.89	2.42	11.9	0.177	
	4:00	Phenolsulphonephthalein injected							
	4:05	Urine collected		0.202	15.48	1.377	6.75	0.091	
	4:37	Sacrificed	110	25% of phenolsul. excreted by left kidney, 23.2 by right					0.206
October 19, wolf	9:35	Operation completed							

October 19, wolf hound; 19 kilos; right kidney. Water by stomach tube throughout	9:35	Operation completed							
	10:30	20 cc. of 20% saline injected							
	10:50	Urine collection begun							
	11:20	Urine collected	90	0.198	8.012	1.514	7.65	0.189	
	11:40	Urine collected	80		7.690	1.91	9.65	0.248	1.392
	12:00	Urine collected	90	0.207	7.692	2.13	10.78	0.277	1.52
	12:01	Caffeine in 20 cc. of 20% saline injected							
	12:15	Urine collected	110		7.992	6.329	31.97	0.792	1.332
	12:37	Urine collected	110	0.204	7.623	5.892	29.76	0.780	1.322
	12:52	Urine collected	90		6.712	6.827	39.00	0.979	1.210
	1:08	Urine collected	96	0.189	6.340	6.45	34.3	1.097	
	1:24	Urine collected	100		7.590	7.24	36.6	0.912	1.280
	1:46	Urine collected		0.194	10.55	7.68	38.8	0.728	1.54
	2:03	Caffeine in 10 cc. of 0.9% saline injected							
	2:05	Urine collected	90	0.195	10.83	5.82	29.4	0.538	1.972
	2:19	Urine collected			9.31	8.48	42.8	0.931	1.632
	2:35	Urine collected	130	0.189	9.64	7.036	35.56	0.729	1.84
	2:50	Urine collected	120	0.206	13.98	8.06	40.76	0.633	1.632
	3:23	Urine collected	120		25.32	7.473	38.9	0.277	2.16
	3:25	Phenolsulphonephthalein injected							
December 11, mongrel; 12.5 kilos, right kidney. Water by stomach tube throughout the experiment	9:10	Operation completed							
	9:55	Injection of 12 cc. of 20% saline	134						
	10:30	Collection of urine begun							
	11:00	Urine collected		0.192	10.53	3.26	17.96	0.309	1.46
	11:30	Urine collected	134		13.80	3.96	21.87	0.384	
	11:43	Caffeine in 20% saline injected							
	11:45	Urine collected			10.35	3.66	20.84	0.345	
	12:00	Urine collected	132	0.174	14.00	4.368	24.83	0.758	1.45
	12:15	Urine collected			10.28	7.45	42.38	0.384	1.46
	12:30	Urine collected		0.169	14.29	5.28	31.21	0.374	1.42
	1:00	Urine collected	124	0.183	15.53	5.56	31.66	0.278	1.35
	1:30	Urine collected	112	0.158	15.77	4.21	23.96	0.268	
	2:00	Urine collected			15.32	3.95	22.46	0.196	1.35
	2:05	Phenolsulphonephthalein injected							
	3:05	Urine collected	80	47.6% of phenolsulph. excreted by right kidney				0.210	

nervated kidney under conditions quite comparable except for the fact that they did not make injections of caffeine. Marshall and Crane obtained a uniform slight increase in rate of elimination of both water and urea on denervating the kidney, which they prove to be due to the increased blood flow. Here there is a marked increase in urea excretion independent of changes in water output in a kidney already denervated. On the other hand, that vasoconstriction is not necessarily an accompaniment of the depressant effects of caffeine is nicely shown in the one experiment in which marked and nearly equal diuresis occurred in both kidneys with definitely increased $\frac{D}{B}$ ratio in the left kidney and an even greater depression of the same ratio in the right kidney.

As has been said, caffeine does not alter the blood in any way which would produce these results, so far as is known. The indications are that caffeine acts specifically on renal epithelium as a stimulus or a depressant, the depression probably depending upon the size of the dose and the condition of the renal epithelium.

Other experiments are in progress designed to solve some of the problems suggested by this work and extending the investigation to other diuretics.

SUMMARY

The results reported in this paper are all derived from crucial experiments performed on dogs with denervated kidneys under veronal anesthesia.

An increased concentration of blood urea and the injection of caffeine alter the rate of excretion of water and of urea as measured in terms of the ratio $\frac{\text{Urea excretion rate}}{\text{Blood urea concentration}}$ independently showing that there is no direct relation between volume and urea output.

Caffeine depresses, leaves unaltered, or augments the rate of excretion of urea. Augmentation is accompanied by a similar increase in elimination of ammonia and by a constant or very slightly lowered depression of freezing point values.

These experiments do not demonstrate whether caffeine produces its effect by vasomotor changes or direct action on the renal epithelium. Vasomotor changes such as would occur in a denervated kidney under the conditions of the experiment would not seem to account for the results obtained, however. It is concluded therefore that caffeine probably acts directly on renal epithelium as a stimulant in some cases, as a depressant in others.

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OBSERVATIONS ON VENOUS PRESSURE AND SKIN BLANCHING PRESSURE BY A MODIFIED METHOD

H. L. WHITE

From the Physiological Department of Washington University, St. Louis

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OBSERVATIONS ON VENOUS PRESSURE. The principle of applying air pressure to a transparent chamber covering a superficial vein to determine the pressure in the vein has been applied by numerous investigators (1), (2), (3), (4), (5). These observers have described various types of apparatus designed to utilize this principle. The variations in the types of apparatus used have been in the style of chamber or capsule employed to cover the vein. In all of them the pressure inside the capsule is raised by manipulation of a small hand pump or rubber bulb, the pressure being gradually raised until the vein collapses. Various modifications of the method for establishing the criterion of intravenous pressure are suggested, such as raising the pressure above that required to collapse the vein and taking as the reading the pressure at which the vein reappears (2) or taking the reading at the point where the vein shadow alternately disappears and reappears with slight oscillations of the pressure (4), (5). A common feature of these procedures is that the pressure increase inside the capsule has obtained for some time, a matter of several seconds at the least, before the reading can be made. A theoretical objection to this method of determining venous pressure may be raised on the grounds that the *vis a tergo* begins to manifest itself in the peripheral segment of the vein immediately the vein is subjected to a collapsing pressure. That is, the pressure in the vein begins to rise immediately when the venous flow is obstructed, with the result that the pressure required to collapse the vein increases, up to a certain limit, with the duration of the application of pressure. It would appear that the ideal arrangement would be the instantaneous application of a collapsing pressure. The lowest pressure which, when instantaneously applied, will produce collapse of the vein, even though that collapse be only momentary, is, neglecting tissue resistance, the true pressure normally existing in the unobstructed vein. We should expect the method utilizing this principle to give lower figures for venous pressure than have previously been reported. The method here described is based upon the application of this principle.

One might reply that this objection is not valid, since the blood which meets an obstruction in a collapsed vein might find a return path to the

heart through anastomotic branches before the pressure in the peripheral segment of the obstructed vein would have an opportunity to rise to an appreciable extent. Whether or not the *vis a tergo* would have an opportunity to manifest itself in the peripheral segment of an obstructed vein would depend upon the facility with which the returning blood could be redistributed to anastomotic channels. The answer to this is the experimentally established fact that the figures for venous pressure obtained on instantaneous application of pressure are considerably lower than those obtained at the same sitting on the same subject with a gradual application of pressure. Furthermore, when the minimum pressure necessary to produce complete collapse is applied instantaneously, the collapse is only momentary, the vein shadow reappearing within one to two seconds. The experimental results thus confirm the theoretical deductions as to the advantage of an instantaneous application of pressure.

Method. The arrangement used for applying pressure to the vein is a metal tank of about 50 liters capacity supplied with stopcocks arranged for connecting the tank with a supply of compressed air, with a water manometer and with the capsule placed over the vein. The arrangement of stopcocks is such that the pressure can be released from the capsule without being released from the tank. The pressure in the tank is raised to some point above that required to collapse the vein and is then lowered in steps of one-half centimeter of water, the pressure being released from the capsule each time by means of the T stopcock inserted between the tank and the capsule, until the lowest pressure which will produce complete collapse of the vein is determined. The whole procedure usually does not take more than 15 seconds. The arrangement of the apparatus is seen in figure 1.

Some details of technique may be given here. The subject sits comfortably in a chair provided with a head rest. In all cases he is permitted to sit quietly for at least five minutes before any determinations are made. The forearm and hand rest on a shelf which can be fixed at any desired height. The mean level of the preventricular reservoir is taken as the level of the bottom of the body of the sternum, the costal angle. This level is marked on the skin and the supporting shelf then fixed at such a height that the level of the vein to be observed coincides with the level of the costal angle. This adjustment is made accurately with a carpenter's spirit level. In this way the necessity of correcting for hydrostatic pressure is avoided.

As stated above, when the minimum pressure necessary to produce complete collapse of the vein is applied the collapse is only momentary. Within one or two seconds the vein shadow reappears, showing that the intravenous pressure has already begun to rise against the obstructing pressure. This is striking evidence of the necessity of using instantane-

ous application of pressure in order to get consistent results. With this method of applying pressure the collapsing pressure is very sharply defined, the end point being easily read to one-half centimeter of water.

Determinations of the pressure in a vein of the dorsum of the hand were made in a series of nine subjects, determinations being made at one sitting on each subject with both gradual and instantaneous pressure application. The glass capsule described by Hooker (4) was used for covering the vein. Gradual application of pressure was effected by means of a small hand pump. The instroke of the piston can be stopped at any point and the resultant pressure will be maintained. A comparison of results obtained by the two methods is given in table 1.

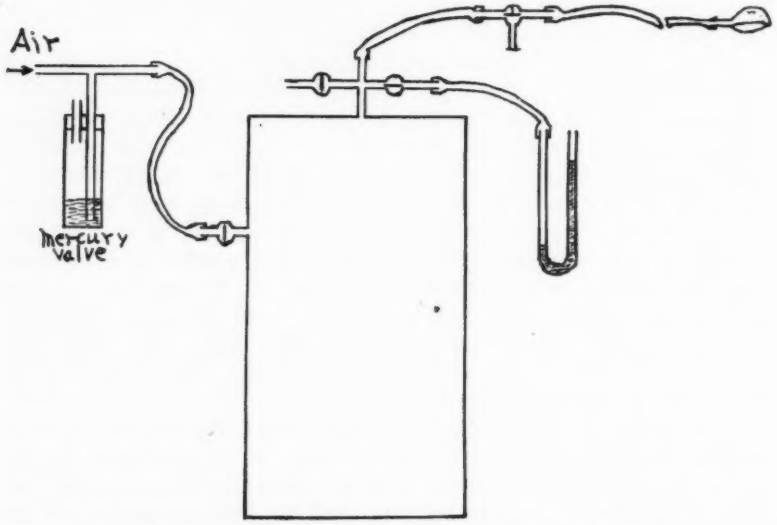

Fig. 1. Apparatus for determining venous pressure and skin blanching pressure.

It is seen that the figures obtained by the method described here are from 1 to 4 cm. of water lower than the figures obtained with the old method. Furthermore the collapsing pressure is much more sharply defined. It is easy to see why figures obtained on consecutive determinations by the old method should vary by as much as 1 to 2 cm. of water. The greater the duration of the application of pressure the higher will be the collapsing pressure. It can easily be shown that the more slowly the pressure is applied the higher it must be raised to bring about complete collapse. In order to get perfectly consistent results with the old method one must be sure that the rate of application of pressure is constant from one determi-

nation to the next; it is obvious that the attainment of this constant rate is practically impossible.

OBSERVATIONS ON SKIN BLANCHING PRESSURE. Danzer and Hooker (6), in a paper in which they describe a method for determining capillary pressure based on direct observation of the capillaries, review the literature of capillary pressure determinations. They conclude that the principle that the pressure required to blanch the skin represents the pressure in the skin capillaries is incorrect and that the skin blanching pressure rather represents the pressure in the skin venules. Since, however, the figures previously reported for skin blanching pressure were obtained by gradual application of pressure and since they exhibit a very wide range of variations it was thought advisable to repeat these determinations, using instantaneous pressure application. A small glass capsule was blown to fit over

TABLE 1

SUBJECT	AGE	VENOUS PRESSURE PRESSURE APPLIED BY		SKIN BLANCHING PRESSURE (CENTIMETERS OF WATER)	DIFFERENCE OF SKIN BLANCHING PRESSURE AND VENOUS PRESSURE (CENTIMETERS OF WATER)
		Hand pump (centimeters of water)	Air tank (centimeters of water)		
	years				
Z	56	8–9	5	24½	19½
He	21	11	9½	13	3½
W	18	4½	3½	9½	6
E	42	4 –4½	3½	19½	16
T	22	7½–8	6	12	6
D	28	4½–5	3½	21½	18
M	24	5 –5½	4	8	4
H	29	4½–5	3½	21	17½
F	25	5 –5½	3½	12	8½

the back of the finger at the base of the nail and sealed on with collodion. The arrangement for applying pressure described in connection with the venous pressure determinations was used. The pressure in the tank is raised to some point above the supposed skin blanching pressure and then allowed to fall slowly, the tank pressure being admitted to and released from the capsule by the T stopcock every one or two seconds while the tank pressure is falling, until the lowest pressure necessary to produce an unmistakable color change in the skin under the capsule is reached. The level of the observed area is made to coincide with the level of the costal angle, these determinations being made on the same subjects and at the same sittings with the venous pressure determinations. It is clear that the term "skin blanching" may mean anything from the first unmistakable change in color to a complete paling. Failure to maintain a uniform criterion has been the chief source of the wide variations in the figures reported.

Since the pressure required to produce complete paling gives excessively high figures, 70 mm. Hg (7), 40 to 50 mm. Hg (8), and since there can be no uniformity in the determination of color changes intermediate between complete paling and the first unmistakable color change, it is obvious that the latter, which was adopted by Basler (9) is the only reasonable criterion and it was the one adopted in these observations. The results are seen in table 1.

It will be noted that the highest skin blanching pressure, 24½ cm. water, was observed in a subject 56 years old. Three other moderately high pressures, 19½, 21, 21½ cm. water were obtained in subjects 42, 29 and 28 years old respectively. The other five subjects ranged in age from 18 to 25 years with pressures ranging from 8 to 13 cm. water. There is no correspondence between the figures representing the difference between skin blanching pressure and venous pressure and those of venous pressure. Thus Z, with a venous pressure of 5 cm. water, showed a difference between skin blanching pressure and venous pressure of 19½ cm. water, while He, with a venous pressure of 9½ cm. water, showed a difference of only 3½ cm. water. If we interpret the skin blanching pressure as being an index of capillary pressure, we are forced to assume that Z has in his circulatory path, interposed between the capillaries and large vein, a resistance amounting to 19½ cm. water while He has in the same region a resistance corresponding to only 3½ cm. water. It seems more reasonable to suppose that the variations in skin blanching pressure are due to variations in skin resistance and that the skin blanching pressure is no adequate index of capillary pressure. Additional evidence in favor of this view is afforded by the fact that of the four individuals, Z, E, D and H, exhibiting the highest skin blanching pressures, the first three are employees of the department whose work is of the kind which would toughen the skin. The fourth, H, is a student and may be supposed to have naturally a more resistant skin than the average. The other five subjects are all students who presumably do very little manual work. The mean age of the four subjects showing the highest skin blanching pressure is 39 years, while the mean age of the other five subjects is 22 years. Whatever relation may exist between age and skin blanching pressure might seem more probably due to the effect of age on skin resistance than to an effect on capillary pressure.

It seemed advisable, however, to try to get more direct evidence than the above as to the significance of the skin blanching pressure. Certain procedures might be carried out and their effect on venous pressure and skin blanching pressure observed. With the help of the data so obtained it would seem that we might more intelligently adjudge the phenomena under observation. The effects of local exercise, cold and heat on the venous pressure and skin blanching pressure of the hand were observed.

Observations on the effect of these three influences were made on a series of subjects, the three factors being investigated at one sitting on any given subject and in the order named. Cold was applied by partially immersing the hand for two to three minutes in water at a temperature of 3° to 5° C., heat by partially immersing the hand for two to three minutes in water at a temperature of 45° to 47° C.; local exercise consisted in alternate flexion and extension of the fingers and thumb of the hand under observation for a period of one minute. The readings were made immediately at the

TABLE 2

All pressures given in centimeters of water

SUBJECT		NORMAL	AFTER EXERCISE	HEAT 45°–47°C.	COLD 3°–5°C.
E	Venous pressure	4	4½	4	4½
	Skin blanching pressure	23	21½	19	19
D	Venous pressure	4½	4½	4½	5½
	Skin blanching pressure	19	13	10½	10
M	Venous pressure	5	5½	5½	6
	Skin blanching pressure	10	8	7	8
Z	Venous pressure	5½	5	6½	5½
	Skin blanching pressure	16½	14½	11½	11½
He	Venous pressure	12½	13½	12½	13
	Skin blanching pressure	12	10	9	10
Mr	Venous pressure	6	7	5½	6½
	Skin blanching pressure	8	7	7½	7½
H	Venous pressure	4½	6	6	6
	Skin blanching pressure	12	9	9	9½
Dav	Venous pressure	6½	8	8	8
	Skin blanching pressure	11	8½	9½	11

cessation of exercise. During the exercise it was necessary to hold the finger with the capsule attached, in order that the seal of the capsule to the finger be not broken.

The problem is to decide whether skin blanching pressure represents capillary pressure or the pressure in the skin venules. If the former is the case we should expect application of heat to bring about a rise in skin blanching pressure since it is definitely established (6) that application of heat raises capillary pressure, apparently through arteriolar dilatation. If, on the other hand, the skin blanching pressure represents the pressure in the skin venules, the effect of application of heat might not be easily pre-

dicted. The rise in capillary pressure might be expected to be in part transmitted to the venules. The venodilatation resulting from heat would, on the other hand, render more easily discernible the color changes in the most superficial layer of the skin venule plexus. This is the point of the criticism made by Danzer and Hooker (6) of Hough and Ballantyne's (8) observations. Thus, if skin blanching pressure represents the pressure in the skin venules, whether or not it would be raised by application of heat would depend upon which of these factors predominates. It may be safely said, however, that if application of heat lowers skin blanching pressure the latter is ruled out as representing capillary pressure. Further points which were borne in mind in the observation of these various influences will be discussed after the results have been given.

As stated above, the normal skin blanching pressures and venous pressures were determined under normal conditions and then immediately after a standard period of local exercise and during a standardized application of heat and of cold. The results are seen in table 2.

It may be stated in passing that heat invariably increased and cold decreased the caliber of the superficial hand veins. This is conclusive proof, if further proof were needed, of a nervous venopressor mechanism.

The interpretation of the results in table 2 involves a consideration of a number of factors. Let us first consider the effect of local exercise. This was used with the idea that the resulting arteriolar dilatation would effect a rise in capillary pressure. If skin blanching pressure represents capillary pressure we might then expect a rise of the former just after local exercise. This would, of course, be true only if the exercise produced a dilatation of the skin arterioles as well as those of the muscles, and there is no conclusive evidence that this is the case with exercise as light as that employed here. However, instead of the possibly expected rise in skin blanching pressure we find a fall. At the same time the venous pressure was either unchanged or somewhat elevated. We may say here that these readings were made within a few seconds, never more than seven or eight, after the cessation of exercise. The skin blanching pressure was determined immediately at the cessation of exercise, the subject rested a few minutes and the venous pressure was then determined with the same time relations to an identical period of exercise. In order to interpret these findings we must consider the effect of the application of the capsule on the color of the skin beneath. When the capsule is applied the skin beneath is invariably redder than that surrounding. This is in all probability due to the fact that the ring of collodion obstructs the circulation in the skin beneath the capsule. Since the venous return is affected more than the arterial entrance, a passive congestion results. We interpret the slight fall in skin blanching pressure on local exercise as follows: On exercise some dilatation of the skin venules takes place with the result that the most

superficial layer of the skin venule plexus is more adequately filled and the reddening of the skin beneath the capsule is accentuated. The first change in color will thus be brought out at a lower pressure than before.

The effect of application of heat is an invariable fall in skin blanching pressure with no change or slight rise in venous pressure. It would be difficult to reconcile these findings with the conception that skin blanching pressure represents capillary pressure. They are readily explainable on the view that the more adequate filling of the most superficial layer of the skin venule plexus renders the color change more readily discernible. It would appear that whatever pressure rise in the skin venules may occur as a result of the capillary dilatation by heat is masked by the opposing effect of the more adequate filling. That some rise may occur is evidenced by the fact that the pressure in the superficial dorsal veins rises slightly in some cases on the application of heat. As is seen, this rise is not a constant phenomenon and is never very great. This does not necessarily mean that an arteriolar dilatation is not of constant occurrence, but may merely mean that whatever tendency toward rise of venous pressure may be brought about by arteriolar dilatation is overcome, partially or completely, by the increase in caliber of the veins which lowers the resistance offered to the venous return.

The results of our observations on the effect of cold on skin blanching pressure were rather surprising. Danzer and Hooker (6) have shown beyond question that cold lowers capillary pressure. If skin blanching pressure represents capillary pressure we should then expect to find the former lowered by cold. If, on the other hand, the skin blanching pressure represents the pressure in the skin venules we should expect to find it lowered by any agency which would increase, and raised by any agency which would decrease, the filling of these venules. In other words, a lowering of skin blanching pressure need not necessarily mean that we are dealing with capillary pressure. To be sure, we had expected to find that cold would bring about a paling of the skin, with a rise in skin blanching pressure if our hypothesis that skin blanching pressure represents the pressure in the skin venules was correct. We found, however, that in most cases our partial immersion of the hand in cold water brought about an actual flushing of the skin at the base of the nails; the "cold bath reaction" was effected while the hand was still in the water. Cold thus lowers the skin blanching pressure for the same reason as does heat, i.e., by effecting a more adequate filling of the superficial layers of the skin venule plexus.

The venous pressure is usually slightly raised by cold. This is apparently due to the unmistakable venoconstriction which raises the resistance offered to the venous return.

The figures of subject He are of special interest for several reasons. In the first place the skin blanching pressure is under all conditions lower than the venous pressure. This alone would cast grave doubts on the hypothesis that the skin blanching pressure represents capillary pressure. Of course it is also difficult to see how the pressure in a large vein of the back of the hand could be higher than that in the venules at the base of the nail. There are two possible answers to this difficulty, both of which may play a part. In the first place, the pressure taken as venous pressure is that which will just produce complete collapse of the vein while the skin blanching pressure is taken as that which produces the first perceptible color change. A pressure insufficient to collapse completely the venules might very well partially empty them with a resultant change in color. In making this suggestion as to a possible factor in the genesis of this apparent anomaly we appreciate the limitation of the extent to which partial collapse can occur without complete collapse. Assuming that with instantaneous pressure application there is no time for a pressure mounting effect and excluding the resistance of the overlying tissue and the vessel wall, i.e., starting from the point at which this resistance has just been overcome, it follows that the application of a pressure just below that in the vessel can effect a decrease in the caliber in the vessel only by the amount represented by the difference in the calibers of the vessel when it is stretched under its normal pressure and when it is just full under atmospheric pressure. For it is apparent that any further increase in applied pressure would suddenly and completely collapse the vessel. Whether or not the percentile increase in the caliber of the venules under their normal pressure over their caliber at atmospheric pressure is sufficient to constitute an appreciable factor in the production of the first perceptible color change we do not know. If it is, our apparent anomaly is due in part merely to the fact that we are not using the same criterion for skin blanching pressure as for venous pressure. Since the criterion employed, the first perceptible color change, is the only one that can be used at all and since it is seen to give such anomalous results it is apparent that the whole principle of drawing any conclusions as to capillary pressure from skin blanching pressure is unsound. A second answer to the difficulty may be that in this particular subject the factor of skin resistance was higher on the back of the hand than at the base of the nail.

The figures of He are of interest for another reason. This subject is seen to have the highest resting venous pressure of any in the series. He is an expert swimmer and the only subject in the group who may be said to be in training. Further observations on trained subjects will be necessary before any conclusions can be drawn as to a relation between high venous pressure and training.

The subjects E, D, M, Z, He and H served as subjects both for the experiments in table 1 and in table 2. It will be noted that the resting venous pressure of any given subject is not necessarily the same in the two tables. Hooker (4) reports a diurnal variation in venous pressure. He finds no change in venous pressure as the result of application of heat or cold. In no case did we get any large pressure change due to heat or cold, the greatest change being $1\frac{1}{2}$ cm. water, but we believe that at least those of 1 cm. water or more are outside our limit of experimental error. We believe that the error of the method when the pressure is applied gradually is 1 to 2 cm. water, while with sudden application of pressure it is not more than $\frac{1}{2}$ cm. water. The somewhat larger experimental error of the method employed by Hooker would account for his failure to obtain positive results on heat and cold application. The variations of the resting venous pressure in the same subjects from table 1 to table 2 are probably due in part to the diurnal rhythm and in part to uncontrolled temperature effects.

It is thus seen that the results of the experiments given in table 2 support the conclusion drawn from the less direct evidence of table 1 that the skin blanching pressure is markedly affected by skin resistance. Variations in normal skin blanching pressure from one subject to another are due largely to variations in the normal skin resistance of different subjects, while variations in skin blanching pressure in one and the same subject due to influences such as temperature change and exercise are due to variations in the "effective skin resistance," i.e., the resistance which must be overcome before the pressure can affect the underlying venules. The variations in this "effective skin resistance" are due, of course, not to any changes in the actual texture of the skin but to variations in the extent of filling of the superficial layers of the skin venule plexus.

SUMMARY

A method of determining venous pressure and skin blanching pressure based on the instantaneous application of pressure is described.

Figures for venous pressure are obtained lower than those obtained with a gradual application of pressure. It is believed that the figures for normal venous pressure given in the text books are considerably too high.

Variations in the caliber of and the pressure in the superficial dorsal hand veins afford unquestionable evidence of a nervous venopressor mechanism.

Additional evidence is presented supporting the conclusion reached by Danzer and Hooker (6) that the skin blanching pressure is not an index of capillary pressure.

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THE EFFECTS OF THYROID AND SOME OTHER ENDOCRINE PRODUCTS ON PARAMECIUM

LORANDE LOSS WOODRUFF AND W. W. SWINGLE

From the Osborn Zoölogical Laboratory, Yale University

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The marked influence of the products of various endocrine glands upon metabolism and growth in the higher animals has, in many cases, been interpreted as indicating a more or less direct and specific effect on cell multiplication. Accordingly, various investigators have turned to unicellular animals as affording a ready means of experimenting directly with single cells; and as a result there are extant several interesting studies on the effects of thyroid and other endocrine products on the Protozoa, in particular on Paramecium.

The first investigation was made in 1908 by Nowikoff (1) who, while studying the chemotactic reactions of Paramecium to thyroid extract, incidentally carried out some experiments to determine the effect of this material on the division rate, and concluded: "Die Resultate des Experimentes sind insofern überraschend, als sie eine ausserst intensive Einwirkung der Schilddrüse auf die Vermehrung der Paramecien zeigen."

The next, as well as the most extensive, studies on the effects of thyroid and other endocrine material on the division rate of Paramecium are those by Shumway. In his first paper (2) he reached the conclusion, from experiments on pedigree lines, that "the effect of thyroid is to increase greatly the rate of division, except at the time when the line is nearing the close of its cycle; and, furthermore, that the effect is not permanent after feeding with thyroid is stopped. Similar treatment with thymus gave negative results." Shumway suggested that the effect of the thyroid is "primarily upon the metabolic activities of the cell;" and that "at the close of the life cycle some element of the protoplasm necessary to digestion is lacking, thus inhibiting the effect of the thyroid." Shumway in his second paper (3) corroborated and extended his earlier conclusions, finding that emulsions of raw thyroids, boiled thyroid, and suspensions of the commercial powder produced a "constant and significant increase of 65 per cent in the rate of division over that observed in the common laboratory medium, hay infusion," the greatest acceleration occurring when the reproductive rate of the control was highest. On the other hand "iodothyrin and iodine failed to produce the thyroid effect," as did likewise "gland suspensions" of thymus, pancreas, spleen, ovary, suprarenal and pituitary body. He

noted that Paramecium ingests and digests particles of the thyroid, and suggested that his results with thyroid feeding are due to the presence of a "remarkably stable hormone in the thyroid which may be classified among the 'dissimilatory' hormones."

Budington and Harvey (4), employing Paramecium and Stylonychia from pedigree cultures, but without daily isolation, tested the effect of thyroid material from animals representing the main classes of vertebrates, since previous workers had employed only mammalian thyroids. They used freshly prepared thyroid glands of fish, frog, turtle, bird and cat, and reached the general conclusion that "thyroid ingredients, no matter from what class of vertebrates the gland be taken, produce essentially the same result when given to ciliate Protozoa as a food or as a factor in the medium in which they live, viz., increased division rate."

Abderhalden and Schiffmann (5) studied the action of various substances, including thyroid material, on the rate of division of Paramecium. From half a dozen experiments they concluded that the rate is markedly increased by the thyroid "opton."

Cori (6) tested the influence of commercial thyroid powder and also thyroxin on the division rate of Paramecium, and stated that "faintly alkaline thyroid extract in hay infusion strongly accelerates" the rate, and that "equivalent concentrations of a solution of thyroxin in hay infusion only very slightly accelerate the rate of multiplication."

Riddle and Torrey (7) gave a summary of experiments with thyroxin on Paramecium, in which they found that the effect of the thyroxin is manifest especially in a slight decrease in the rate of division, and a large increase in excretory activity as indicated by an increased rate of pulsation of the contractile vacuoles and a great decrease in the number of excretory crystals in the endoplasm. Accordingly, they concluded that thyroxin appears to promote katabolic rather than anabolic processes.

Before the publication of the papers by Cori and by Riddle and Torrey we had carried on a series of experiments with various endocrine products and reached the conclusion that "neither thyroxin nor commercial desiccated thyroid nor fresh desiccated thyroid of the turtle produce any significant acceleration of the division rate of Paramecium." This was published in brief form (8). We are glad to note that the conclusions of Riddle and Torrey and our own are apparently in substantial agreement in regard to thyroxin. Together they are opposed to the results of all previous investigators of the problem. Since our preliminary communication was published we have carried on more experiments, and the present paper affords the complete data from the entire study.

METHODS. The indication of subtle alterations in the metabolism of a higher animal produced, for instance, by the feeding or the injection of an extract, is obviously complicated by the highly specialized physiological

processes of the animal, as well as by its individual idiosyncrasies. Furthermore, of course, marked differences exist in the action of various substances toward the various tissues as well as toward the same tissue under different conditions. When, however, the organism is reduced to the lowest term, the single cell, these complications are practically eliminated. It is true, to be sure, that the effects produced by extracts, etc., on unicellular forms, may not always afford an exact picture of the effects of these substances upon the specialized cells of the vertebrate. It remains true, however, that a closer approach to the fundamental effects of the substances in question on the basic phenomena of protoplasm should be more readily attained in the Protozoa than in higher forms.

Accordingly, in the present studies, Paramecium was adopted as our "biological reagent" (9) because the ease with which it lends itself to experimental methods makes it one of the most favorable Protozoa for general physiological study, and because we had at our disposal a pedigree race of *Paramecium aurelia* which had been under observation for over fifteen years, and through more than ten thousand generations by cell division (10). This pedigree race has afforded an unfailing supply of Protozoa whose morphology and physiology are accurately known through a series of studies from this laboratory since 1907. Of importance from the standpoint of the problem in hand is the fact that it has been shown that the rate of division of this race is a remarkably accurate indication of the character and temperature of the culture medium; since very slight variations produce significant responses by the organisms. In brief, the reproductive rate may be regarded as essentially a function of the cell's surroundings (11). Furthermore, since all the Paramecia of the race were originally derived from one animal, all the studies were made on "sister" cells, and therefore on the "same protoplasm."

It is necessary to emphasize these facts because it has been shown that the reactions of Paramecia to various substances may be greatly changed by untoward environmental conditions either past or present. In brief, Paramecia pedigreed under conditions which have been demonstrated to be favorable are a *sine qua non* if the animals are to be successfully employed as a "biological reagent."

In preparation for each experiment a single Paramecium was isolated from the main line of the pedigree race with a capillary pipet and placed in a watch glass with a small amount of the regular culture medium, consisting of our standard beef extract. When in the course of a few days the isolated animal had produced by division a sufficient number of cells to start the various lines of the experiment, each animal was isolated on a clean depression slide and supplied either with standard beef extract or with this medium modified with the substance to be tested.

The basic culture medium of beef extract has been the standard medium employed for the culture of Paramecium under consideration and for cultures of other Infusoria in this laboratory since it was formulated after careful experiments made over twelve years ago (12). It has stood the test of time. As usual, for the present experiments a quantity of this solution was made up in water distilled in glass, placed in test tubes and sterilized. Each tube contained sufficient medium for one day's use. Accordingly, the basic culture medium was identical throughout each and every experiment.

The series of depression slides were arranged in moist chambers to prevent evaporation. At the start of the experiment, and also each day, cross-infection was effected, which it is believed rendered the initial daily bacterial flora supplied as food for the Paramecia essentially identical on the various slides. The temperature was maintained at about 23°C., which experiments have shown to be within the optimum zone for this race of Paramecium, but since all the cultures whose division rate is directly compared were carried on synchronously, such slight fluctuations as occurred affected all at the same time and therefore play no significant part in the results.

Every substance in each experiment was tested on four lines of sister cells for ten days. Every day a single animal was isolated from each line, placed on a clean depression slide, and supplied with about 0.3 cc. of fresh medium. At the time of isolation a record was made of the number of divisions which had occurred in each line during the previous twenty-four hours, and these data afford the basis for the results given in the present paper.

In carrying the experiments enthusiastic assistance has been rendered by Miss Hope Spencer of this Laboratory.

Experiment I. The first experiment was planned to show the effect of thyroxin on the division rate of Paramecium. After a number of preliminary tests to determine approximately the greatest concentration of thyroxin which did not too greatly depress the division rate, it was decided to study first the effect of 0.004 per cent and 0.0026 per cent. In view of the fact that thyroxin is practically insoluble in water unless slightly alkaline, NaOH was added: 0.0066 per cent to the former and 0.0044 per cent to the latter. Accordingly, each day sterile beef extract was made up with thyroxin and NaOH in the amounts just stated. In order to determine the effect of NaOH alone and to act as a control for the NaOH necessarily present in the thyroxin solution, there were also made up each day media of standard beef extract with the same percentages of NaOH, viz., with 0.0066 per cent NaOH and with 0.0044 per cent NaOH. Finally, there was the unmodified standard beef extract medium as a control for all.

The definitive experiment extended from November 2 to 11, 1922, and comprised twenty lines of Paramecium: four lines being bred on each of the media described above, which may be referred to as standard beef extract, NaOH (weak), NaOH (strong), thyroxin (weak), and thyroxin (strong). In addition, our basic solution of thyroxin was injected into tadpoles, and its efficacy was proved by markedly accelerating metamorphosis as compared with control tadpoles not so injected.

The following table briefly presents the entire data, giving, in order, the number of divisions in the four lines of Paramecia of each series during the first five-day period; the same during the second five-day period; the total number of divisions for the ten days of the experiment; the number of divisions the thyroxin and the NaOH lines are above or below the standard beef extract control at the end of ten days; and finally, the average daily division rate of the four lines of each culture for the entire ten days of the experiment.

TABLE 1

	DIVISIONS FIRST PERIOD	DIVISIONS SECOND PERIOD	TOTAL DIVISIONS	DIVISIONS ABOVE OR BELOW STANDARD BEEF EXTRACT	AVERAGE DAILY DIVISION RATE FOR 10 DAYS
Standard beef extract	13	14	27		0.67
NaOH, 0.0044 per cent	14	15	29	+2	0.72
NaOH, 0.0066 per cent	16	15	31	+4	0.77
Thyroxin,* 0.0026 per cent	12	4	16	−11	0.40
Thyroxin,* 0.004 per cent	14	1	15	−12	0.37

* In this and the following tables, the percentage of NaOH with the thyroxin has not been indicated since it is the same as in the NaOH controls.

The data in column 5 of table 1 show that both of the NaOH cultures divided slightly more rapidly than the beef control and that both of the thyroxin cultures divided less rapidly than either the NaOH cultures or the beef culture. But long experience with this race of Paramecium has shown that a variation of 0.15 in the average daily rate of division of four lines bred for ten days may be accepted as within the limits of error of the method. Accordingly there is no significant difference between the rates in standard beef extract, NaOH (weak), and NaOH (strong), but there is a significantly lower division rate in both thyroxin media. Accordingly, this experiment indicates in a clear-cut way that the NaOH unavoidably present in the thyroxin media has no appreciable effect, and that 0.0026 per cent and also 0.004 per cent thyroxin in the culture medium produce a significant lowering of the division rate.

Experiment II. This experiment was carried from November 13 to 23, 1922, and is a repetition of the former, except that the concentrations of thyroxin, and therefore of NaOH, employed were lower than in experiment I.

Table 2 gives the data in detail, and shows that 0.0013 per cent and 0.002 per cent solutions of thyroxin produce a significant lowering of the rate of division; the stronger acting as a greater depressant than the weaker solution, and thus agreeing with the results with thyroxin in the former experiment. Furthermore, the data show that 0.0022 per cent and 0.0033 per cent solutions of NaOH produce a lowering of the division rate, the weaker solution producing a very slightly greater effect. Since both the NaOH solutions are weaker than those used in the former experiment, it is evident that these NaOH results do not corroborate those in experiment I. Taking the data from the present experiment alone, one would

TABLE 2

	DIVISIONS FIRST PERIOD	DIVISIONS SECOND PERIOD	TOTAL DIVISIONS	DIVISIONS ABOVE OR BELOW STANDARD BEEF EXTRACT	AVERAGE DAILY DIVISION RATE FOR 10 DAYS
Standard beef extract	24	23	47		1.17
NaOH, 0.0022 per cent	17	13	30	−17	0.75
NaOH, 0.0033 per cent	24	12	36	−11	0.90
Thyroxin, 0.0013 per cent	11	20	31	−16	0.77
Thyroxin, 0.002 per cent	15	4	19	−28	0.47

TABLE 3

	DIVISIONS FIRST PERIOD	DIVISIONS SECOND PERIOD	TOTAL DIVISIONS	DIVISIONS ABOVE OR BELOW STANDARD BEEF EXTRACT	AVERAGE DAILY DIVISION RATE FOR 10 DAYS
Standard beef extract	20	31	51		1.27
NaOH, 0.0011 per cent	15	37	52	+1	1.30
NaOH, 0.00165 per cent	24	27	51	0	1.27
Thyroxin, 0.00065 per cent	21	21	42	−9	1.05
Thyroxin, 0.001 per cent	12	21	33	−18	0.82

be justified in concluding that the depressant effect of thyroxin is due in part to the unavoidable presence in these solutions of NaOH, but it should be emphasized that the strong thyroxin medium, containing the same amount of NaOH as the strong NaOH medium, produced a markedly greater depressant effect than the latter. Obviously, further data are necessary to clear up this point definitely, but the net result from experiments I and II is that thyroxin, in the concentrations tested, lowers the division rate.

Experiment III. From November 27 to December 7, 1922, a third experiment was carried out which is a repetition of the former two, except that the concentrations employed were lower. A survey of the data given

in table 3 corroborates entirely, at lower concentrations, those given in tables 1 and 2 in regard to the depressant effect of thyroxin, and the data in table 1 in regard to NaOH. Thus far, then, the depressant effect produced by NaOH in experiment II remains unique.

Experiment IV. Another repetition of the earlier experiments was carried from December 9 to 19, 1922, but still lower concentrations were used. In addition, lines of Paramecia were tested on standard beef extract

TABLE 4

	DIVISIONS FIRST PERIOD	DIVISIONS SECOND PERIOD	TOTAL DIVISIONS	DIVISIONS ABOVE OR BELOW STANDARD BEEF EXTRACT	AVERAGE DAILY DIVISION RATE FOR 10 DAYS
Standard beef extract	26	26	52		1.30
NaOH, 0.00027 per cent	31	22	53	+1	1.32
NaOH, 0.00041 per cent	31	24	55	+2	1.37
Thyroxin, 0.00016 per cent	30	28	58	+6	1.45
Thyroxin, 0.00025 per cent	27	24	51	−1	1.27
Thyroid	19	11	30	−22	0.75
Pituitary	36	28	64	+12	1.60
Pineal	35	25	60	+10	1.50

TABLE 5

	DIVISIONS FIRST PERIOD	DIVISIONS SECOND PERIOD	TOTAL DIVISIONS	DIVISIONS ABOVE OR BELOW STANDARD BEEF EXTRACT	AVERAGE DAILY DIVISION RATE FOR 10 DAYS
Standard beef extract	18	22	40		1.00
NaOH, 0.000165 per cent	15	19	34	−6	0.85
NaOH, 0.00165 per cent	19	20	39	−1	0.97
Thyroxin, 0.0001 per cent	16	18	34	−6	0.85
Thyroxin, 0.001 per cent	15	14	29	−11	0.72
Thyroid	21	18	39	−1	0.97
Pituitary	18	25	43	+3	1.07
Pineal	20	23	43	+3	1.07

medium to which was added commercial desiccated beef thyroid, pituitary, and pineal material, respectively. The beef thyroid material used in this and subsequent experiments was proved to be highly active in accelerating metamophisis of thyroidectomized and hypophysectomized tadpoles (13).

The complete data are presented in table 4. Thyroxin and NaOH obviously produced no significant effect, such variations as there are from the standard beef extract control being within the limits of experimental error. Accordingly, it appears that we have now reached concentrations just below the threshold of effect. The data in regard to the thyroid

medium show a depression of the division rate, while those from the pituitary and the pineal media show stimulation of the division rate, judged by the standard beef extract control.

Experiment V. This series of eight cultures was bred from February 19 to March 2, 1923. As a whole, it is essentially a repetition of experiment IV, while the first five cultures also constitute a repetition of experiments I, II and III.

TABLE 6

	DIVISIONS FIRST PERIOD	DIVISIONS SECOND PERIOD	TOTAL DIVISIONS	DIVISIONS ABOVE OR BELOW STANDARD BEEF EXTRACT	AVERAGE DAILY DIVISION RATE FOR 10 DAYS
Standard beef extract	20	21	41		1.02
NaOH, 0.00033 per cent	20	14	34	−7	0.85
NaOH, 0.0033 per cent	18	19	37	−4	0.92
Thyroxin, 0.0002 per cent	19	12	31	−10	0.77
Thyroxin, 0.002 per cent	21	14	35	−6	0.87
Pituitary	24	26	50	+9	1.25
Thyroid (commercial)	17	27	44	+3	1.10
Pineal	23	21	44	+3	1.10
Turtle thyroid	31	32	63	+22	1.57
Turtle leg muscle	30	33	63	+22	1.57

TABLE 7

	DIVISIONS FIRST PERIOD	DIVISIONS SECOND PERIOD	TOTAL DIVISIONS	DIVISIONS ABOVE OR BELOW STANDARD BEEF EXTRACT	AVERAGE DAILY DIVISION RATE FOR 10 DAYS
Standard beef extract	16	10	26		0.65
NaOH, 0.00165 per cent	17	13	30	+4	0.75
Thyroxin, 0.0005 per cent	14	13	27	+1	0.67
Thyroxin and pituitary	20	31	51	+25	1.27
Pituitary	24	28	52	+26	1.30
Pineal	24	26	50	+24	1.20

The data show no effect from either 0.00165 per cent or 0.000165 per cent NaOH. Those from thyroxin indicate that this substance, in 0.001 per cent solution, lowers the rate of division, which corroborates the result with the same percentage in experiment III; while in 0.0001 per cent solution it produces no significant effect.

Analysis of the data from standard beef media to which desiccated glandular material has been added shows that neither thyroid, pituitary, or pineal produced any significant effect on the rate of division. This is in striking contrast with the results given in table 4. More data clearly are needed on this point.

Experiment VI. This experiment extended from March 8 to 18, 1923, and consisted of ten cultures. It is practically a repetition of experiment V, though different concentrations of NaOH and thyroxin were tested and two other media were added: freshly desiccated thyroid and leg muscle of the turtle. The turtle thyroid material used in this and subsequent experiments was proved to be highly active in accelerating metamorphosis of thyroidectomized and hypophysectomized tadpoles (13).

Table 6 presents the entire data. It will be noted that both thyroxin and NaOH (strong) did not significantly change the division rate, whereas both thyroxin and NaOH (weak) produced a lowering which should be significant according to our standard. No explanation is apparent for this slightly aberrant result, but it does not obscure the main fact that thyroxin either has no effect or a depressant effect on the division rate. None of the cultures bred in standard beef extract plus commercial desiccated glandular material showed any difference in division rate from that of the standard beef medium, except the pituitary which slightly accelerated the rate. Therefore, the thyroid and pineal data agree with those obtained in experiment V, while the pituitary data agree with those in experiment IV. The series carried on the freshly desiccated turtle thyroid and turtle leg muscle both showed a marked, and, it so happened, an identical accelerating effect on the division rate.

Thus far, therefore, the results with desiccated glandular products in the different experiments show a lack of agreement which is in striking contrast to those from thyroxin and NaOH. But the explanation is not far to seek. The former introduce unavoidable variable factors, the most important of which is changes in the food supply of the Paramecia.

Experiment VII. This series of cultures was conducted from December 28, 1923, to January 7, 1924, and consists essentially of a study of a combination of thyroxin with fresh pituitary material from the frog. Table 7 shows that the results both with thyroxin and with NaOH are concordant with the general results of the previous experiments. Fresh pituitary exactly doubled the rate of division. Thyroxin added to the pituitary medium produced no significant change from that of pituitary alone. The desiccated pineal gland showed a marked acceleration of the rate as compared with the standard beef medium, but no significant difference from the pituitary with or without thyroxin. Obviously, then, the effect of thyroxin in combination with pituitary is not different from thyroxin alone in similar concentrations in standard beef medium. The results from fresh pituitary material agree with those from all the former tests with desiccated material except experiment V; while those from pineal agree thus far only with those of experiment IV. However, it should be noted, neither pituitary nor pineal have ever lowered the rate of division below that of the standard beef control; their action invariably being either neutral or accelerating.

Experiments VIII and IX. These two experiments were carried simultaneously, from January 18 to 28, 1924, not only to supply further data, but chiefly to afford a final demonstration of the precision of the experimental procedure used throughout this study. Obviously, since all the cultures lived synchronously, they were subjected to identical temperature and other environmental conditions, and also the animals in each culture were in the same "physiological condition." Such being the case, the similar cultures in each, as well as the two experiments as a whole, act as reciprocal controls.

TABLE 8

	DIVISIONS FIRST PERIOD	DIVISIONS SECOND PERIOD	TOTAL DIVISIONS	DIVISIONS ABOVE OR BELOW STANDARD BEEF EXTRACT	AVERAGE DAILY DIVISION RATE FOR 10 DAYS
Standard beef extract	24	22	46		1.15
Muscle	33	23	56	+10	1.40
Thyroid	30	28	58	+12	1.45
Pineal	30	24	54	+8	1.35
Pituitary	36	23	59	+13	1.47

TABLE 9

	DIVISIONS FIRST PERIOD	DIVISIONS SECOND PERIOD	TOTAL DIVISIONS	DIVISIONS ABOVE OR BELOW STANDARD BEEF EXTRACT	AVERAGE DAILY DIVISION RATE FOR 10 DAYS
Standard beef extract	27	17	44		1.10
Muscle	34	24	58	+14	1.45
Thyroid	36	22	58	+14	1.45
Pineal	28	22	50	+6	1.25
Pituitary	36	26	62	+18	1.55

Inspection of the data from comparable cultures given in column 5 of table 8 and table 9 discloses that the greatest variation in the average division rate of the four lines for ten days is 0.10, and in one case there is no variation at all. This clearly shows the reliability of the method and that the 0.15 limit of error which we have used in interpreting the data is just.

Studying the same tables from the standpoint of the comparative effects of the material employed, it is apparent that fresh desiccated beef muscle, and commercial desiccated thyroid, pituitary, and pineal material consistently give a slightly higher division rate than the standard beef medium alone, though in one instance the pineal acceleration can not be regarded as significant since it exactly coincides with the limit of error of the method. The thyroid data indicate a slight acceleration of the division rate and so agree only with those from fresh thyroid in experiment VI. The pituitary

data agree with those from all former experiments except experiment V. The pineal data from experiment IX, since they show an acceleration in the rate which is just too small to be significant, and accordingly do not agree with the data synchronously supplied by experiment VIII, afford the sole inconsistency in this final test experiment.

SURVEY OF THE RESULTS OF ALL THE EXPERIMENTS. A synoptic survey of the essential data from all the experiments in regard to thyroxin and NaOH is presented in table 10. From inspection of this table it is evident that of the thirteen cultures tested in regard to sodium hydroxide, ten cultures showed no significant effect, the variation from the standard beef

TABLE 10

	EXPERIMENT I	EXPERIMENT II	EXPERIMENT III	EXPERIMENT IV	EXPERIMENT V	EXPERIMENT VI	EXPERIMENT VII
Sodium hydroxide	0.0044%	0.0022%	0.0011%	0.00027%	0.000165%	0.00033%	0.00165%
	0	—	0	0	0	—	0
Sodium hydroxide	0.0066%	0.0033%	0.00165%	0.00041%	0.00165%	0.0033%	
	0	—	0	0	0	0	
Thyroxin + sodium hydroxide	0.0026%	0.0013%	0.00065%	0.00016%	0.0001%	0.0002%	0.0005%
	0.0044%	0.0022%	0.0011%	0.00027%	0.000165%	0.00033%	0.00165%
	—	—	—	0	0	—	0
Thyroxin + sodium hydroxide	0.004%	0.002%	0.001%	0.00025%	0.001%	0.002%	
	0.0066%	0.0033%	0.00165%	0.00041%	0.00165%	0.0033%	
	—	—	—	0	—	0	

extract control being within the limit of error of the method. Three cultures showed a slight but significant lowering of the division rate. Two of the latter occur in the same experiment (II) and in a solution intermediate in concentration between others which clearly show no effect. Furthermore, the data for the first five days of the experiment (cf. table 2) indicate little or no effect, and only during the second five days is the rate markedly lowered. The remaining culture showing depression was subjected to a very low concentration and was carried synchronously with another culture in experiment VI of much greater concentration which showed no significant effect.

Accordingly, the three cultures indicating that sodium hydroxide (in concentrations present in association with thyroxin) produces a lowering of the division rate are obviously exceptions for which no explanation is apparent. The remaining ten cultures, at widely varying concentrations, definitely establish that the sodium hydroxide necessarily present in the thyroxin cultures has no significant influence, and accordingly that whatever changes in the rate of division the thyroxin cultures reveal, certainly are not attributable to the associated NaOH.

We may turn now to a comparison of the effects of standard beef extract media with and without thyroxin. Inspection of table 10 shows that thirteen cultures were subjected to thyroxin in various concentrations. Eight of these exhibited a lower division rate than that of their respective standard beef extract control. Five of the eight exhibited a lower rate of division than their respective standard beef extract plus sodium hydroxide control. Not a single culture divided at a significantly higher rate than the control.

The lower concentrations of thyroxin, in general, produce no change in the division rate, thus showing that the percentages tested range above and below the threshold of influence of thyroxin under the conditions of the experiment. In brief, the concordant results derived from these experiments afford no support whatsoever for the view that thyroxin accelerates cell division in Paramecium, but, on the contrary, show that, in sufficient concentrations, it depresses cell division in Paramecium.

TABLE 11

	EXPERIMENT IV	EXPERIMENT V	EXPERIMENT VI	EXPERIMENT VII	EXPERIMENT VIII	EXPERIMENT IX
Muscle (beef)					+	+
Muscle (turtle)			+			
Thyroid (commercial)	−	0	0		+	+
Thyroid (turtle)			+			
Pituitary	+	0	+	+	+	+
Pineal	+	0	0	+	+	0

Having shown that thyroxin does not accelerate the rate of division of Paramecium, we may turn to a synoptic view of the data in regard to the effect of desiccated glandular material when added to the standard beef extract medium. In this study there are two unavoidable complications which are not present in the thyroxin and NaOH work, viz., *1*, impossibility of supplying to each culture exactly the same amount of the desiccated material; *2*, alteration of the food supply of the Paramecia, since the glandular material supplies more food for both the Paramecia and for the Bacteria, and therefore the latter multiply more rapidly and afford still more food for the Paramecia. Accordingly, standard beef extract without desiccated glandular material is not an adequate control for beef extract with desiccated glandular material.

However, it is worth while to review the results which are summarized in table 11. Inspection of this table shows that desiccated thyroid (beef commercial and turtle) was tested in six cultures. Three times it produced an acceleration, once a depression, and twice no effect on the division rate as compared with the respective controls on standard beef. Obviously, no general conclusion can be drawn, though, as is to be expected,

the weight of evidence decidedly favors acceleration, when it is borne in mind that the thyroxin content would, if anything, tend to neutralize the acceleration due to increase of food supply of the Paramecia.

A real test of the effect of desiccated thyroid material, however, is afforded when desiccated muscle tissue is employed as a control. Such data are supplied in tables 6, 8 and 9 and show that, in three out of four cultures, thyroid produced no significant alteration in the division rate, and in the fourth culture it produced a lowering of the rate. This result, especially when the details presented in the tables are analysed, shows conclusively that thyroid tissue does not produce a higher division rate than muscle tissue. Or, combining these data with those discussed in the previous paragraph, we may state the same conclusion in another way: using standard beef extract as the control, solutions of desiccated beef tissue accelerate the division rate more than do solutions of desiccated thyroid tissue.

In order to make doubly secure the conclusion that thyroid possesses no stimulating properties peculiar to itself, controls of desiccated pituitary and pineal materials were also employed for the thyroid. Inspection of table 11 shows that both pituitary and pineal invariably either accelerated or produced no effect on the division rate with standard beef as a control. No depression ever resulted. This might be anticipated for the reasons stated above in regard to thyroid. Furthermore, a comparison of tables 4, 5, 6, 8 and 9 shows that of six cultures in which pituitary was a control for thyroid, one indicates acceleration by thyroid, one indicates depression by thyroid, and four indicate no change due to thyroid. Finally, the same tables show that of six cultures for which pineal was a control, two indicate acceleration by thyroid, one indicates depression by thyroid, and three indicate no change due to thyroid.

In brief, the experiments which compare the division rate of animals bred in solutions containing thyroid material with the division rate of animals bred in solutions containing muscle, pituitary, or pineal material, collectively show that thyroid material possesses intrinsically no stimulating property peculiar to itself. Such apparent isolated exceptions to this generalization as appear are clearly explained by the fact that culture media containing thyroid tissue, and likewise those containing muscle, pituitary, or pineal tissue, afford, both directly, and indirectly through bacteria, varying amounts of extra food material.

CONCLUSIONS

No purpose will be served by discussing in detail the experiments of previous workers who have found that thyroid and other endocrine products possess an intrinsic property which stimulates cell division in Paramecium. The crux of the matter, we believe, rests on the character of the controls.

Thyroid and other glandular material supply directly and indirectly more food, and accordingly, for significant results, this factor must be taken into account in devising the controls. It is not clear that previous workers have given this due consideration.

Turning to thyroxin, the generally accepted active principle of the thyroid gland, which has been recently isolated and therefore was not available to the earlier workers, the problem of the control is not so difficult since thyroxin does not alter the food content when added to the basic culture medium. The results of Cori indicate a slight acceleration, though, as he stated, not at all proportional to that induced by thyroid material. Riddle and Torrey, however, obtained depression with thyroxin, and our results accord.

The data supplied by the present study show unequivocally, we believe, that neither thyroid, pineal, nor pituitary material possess intrinsic properties which accelerate cell division in Paramecium. Furthermore, thyroxin does not accelerate cell division in Paramecium. On the contrary, above certain concentrations it depresses cell division in Paramecium.

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ON THE RATE OF ELIMINATION OF INORGANIC SALTS FROM THE BLOOD STREAM

OTAKAR BARKUS

Trudeau, N. Y.

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It has been shown in a previous paper (1) that irradiation with ultra violet and sunlight accelerates the rate with which intravenously injected phosphates and sulphates disappear from the blood stream. In connection with this work it has also been found that whereas the elimination of phosphates and sulphates from the blood extended over a considerable length of time, the disappearance of intravenously injected sodium chloride was very rapid; so rapid indeed that the influence of irradiation on the rate of elimination of chlorides from the blood could not be studied. This difference in the behavior of phosphates and sulphates in one instance and of chlorides in the other seemed interesting enough to warrant a more detailed study.

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Experimental. Fullgrown female sheep were used for these experiments. They were fed on an oats and hay diet and received no food or water eighteen hours previous to the experiment; in order to keep the animal quiet 16.2 mgm. morphine sulphate were given subcutaneously before each experiment, and another 16.2 mgm. after three hours. In the experiments where the disappearance from the blood stream of chlorides was followed solutions of 1 per cent, 10 per cent and 35 per cent sodium chloride in 150 cc. water were given; in the experiments with phosphates a dose of 26.0 mgm. P per kgm. in the form of a 12 per cent disodium phosphate solution adjusted to pH 7.3 was infused; in the experiments with sulphates, 27.0 mgm. S per kgm. in the form of a 9 per cent magnesium sulphate solution adjusted to pH 7.3 was administered; this was preceded by infusion of 45 cc. of a 3 per cent calcium chloride solution. The vena femoralis was exposed under local anesthesia (2 per cent novocain) and the solution kept to 30 per cent infused by gravity; the infusion of 125 to 150 cc. was completed in about three minutes; the sheep was then

bled from the same vein at intervals of fifteen, thirty and sixty minutes, and the samples analyzed immediately in triplicate by the methods of Doisy, Denis and Volhardt-Wetmore respectively.

In the experiments where the urinary excretion of phosphates was observed the following technique was used: the animal received morphine sulphate and was tied down as in previous experiments; a short (female) metal catheter was inserted and the bladder emptied by pressure on the abdomen; the catheter was kept in place by means of adhesive tape; the salt solution was then infused as usual and the collecting flasks attached to the table, changed at intervals of thirty and sixty minutes. The volume of each specimen was recorded and the amount of phosphate determined according to Jones.

Three experiments were performed to study the disappearance from the blood stream of intravenously injected chlorides; the data are presented in table 1.

It will be seen from these experiments that the chlorine-ion leaves the blood stream very rapidly; this is especially evident in the experiment 3, where a total of 52.5 grams of NaCl was introduced.

In the experiments covering the excretion of phosphates, different results were obtained in that *the elimination from the blood of phosphates took a much longer time than the elimination of chlorides.* The protocols of these experiments are given below.

It will be noticed from the experiments recorded in table 2, that a comparatively small amount of phosphorus was introduced, and that a large part of it was retained in the blood for a considerable length of time. In order to see how much of the injected phosphate could be recovered from the urine during the time that a retention in the blood of phosphorus was observed, three experiments were performed in which the urinary excretion of phosphorus was followed.

Table 3 shows that only 19 to 22 per cent of the phosphorus introduced reappears in the urine within four hundred and twenty minutes.

A similar behavior as in the excretion of phosphates was observed with intravenously injected sulfates. Two experiments are recorded below.

It will be seen from table 4 that the retention in the blood stream of intravenously injected sulfates is very marked. The toxicity of the small dose employed is negligible when counteracted by calcium chloride; *how far tissue permeability toward sulfates is changed by this measure remains to be determined.*

Three experiments were undertaken to estimate the concentration ratio, i.e., $\frac{\text{urine P Conc}}{\text{plasma P Conc}}$ after the intravenous administration of phosphates; a dose of 0.5 gram P in the form of disodium phosphate was given. The results of these experiments are recorded below.

These experiments show that the concentration ratio is high only in the first period of ninety minutes, after which a drop occurs indicative of retention; and a definite rise in the concentration ratio after this second period of one hundred and eighty minutes.

TABLE 1

TIME AFTER INFUSION			CONCENTRATION NaCl MGM. PER 100 CC. PLASMA		
Experiment 1	Experiment 2	Experiment 3	Experiment 1	Experiment 2	Experiment 3
minutes	*minutes*	*minutes*			
Normal	Normal	Normal	791	770	797
15	10	20	791	818	873
30	30	30	791	810	864
45	40	40	791	776	843

Experiment 1: Sheep, Bl. 1; weight 45.3 kgm. infused with sodium chloride 1 per cent.

Experiment 2: Sheep, Bl. 2; weight 44.6 kgm. infused with sodium chloride 10 per cent.

Experiment 3 Sheep, Bl. 3; weight 39.0 kgm. infused with sodium chloride 35 per cent.

TABLE 2

TIME AFTER INFUSION			CONCENTRATION P MGM. PER 100 CC. PLASMA		
Experiment 1	Experiment 2	Experiment 3	Experiment 1	Experiment 2	Expe iment 3
minutes	*minutes*	*minutes*			
Normal	Normal	Normal	5.8	4.7	5.1
15	15	15	14.6	13.4	13.4
30	30	30	12.1	13.8	12.9
45	45	45	11.2	13.2	10.9
65	65	60	Lost	10.1	10.8
100	95	90	9.2	9.3	8.6
130	125	135	8.0	9.1	7.7
160	155	165	7.1	8.4	7.1
190	185		6.0	8.0	
250	215	225	5.5	5.2	5.5
310			5.3		

Experiment 1: Sheep, Bl. 1.
Experiment 2: Sheep, Bl. 2.
Experiment 3: Sheep, Bl. 3; all infused with 26.0 mgm. P per kgm.

Discussion of results. Denis (2) reported experiments in which phosphates and sulphates were introduced into dogs and rabbits either intravenously or into an intestinal loop; the blood of these animals was then analyzed for the anion in question in intervals of 60 minutes. Denis found that as much as 106 mgm. of P per kgm. when injected intravenously

did not increase the plasma-phosphorus concentration whereas sulfates were markedly retained. Denis concluded that this different behavior of phosphates and sulfates as regards their disappearance from the blood stream is due to a selective action of the kidney. Our experiments with small doses of sulfates confirm Denis' observation in the case of sulfates.

TABLE 3

TIME AFTER INFUSION			VOLUME OF URINE SPECIMEN			GRAM P IN SPECIMEN		
Experiment 1	Experiment 2	Experiment 3	Experiment 1	Experiment 2	Experiment 3	Experiment 1	Experiment 2	Experiment 3
minutes	*minutes*	*minutes*	*cc.*	*cc.*	*cc.*			
30	30	30	55	158	117	0.1313	0.1586	0.1249
60	60	60	18	49	113	0.2006	Lost	0.1386
120	90	90	33	35	78	0.0109	0.0546	0.0661
180	120	120	38	28	29	0.0147	0.0263	0.0284
240	180	180	48	48	105	0.0131	0.0275	0.0284
300	240	240	91	28	130	0.0134	0.0062	0.073
420	360	460	47	71	185	0.0024	0.0063	0.0103
Total.....			330	417	757	0.3864		0.4040

Experiment 1: Sheep, Bl. 3; received a total of 1.84 grams P; 21.05 per cent of the phosphorus infused was excreted.

Experiment 2: Sheep, Br. 2; received a total of 2.16 grams P; 22.2 per cent of the phosphorus infused was excreted.

Experiment 3: Sheep, R. 1; received a total of 2.05 grams P; 19.71 per cent of the phosphorus infused was excreted.

TABLE 4

TIME AFTER INFUSION		CONCENTRATION S MGM. PER 100 CC. WHOLE BLOOD	
Experiment 1	Experiment 2	Experiment 1	Experiment 2
minutes	*minutes*		
Normal	Normal	3.1	3.4
30	30	6.0	8.0
90	90	6.0	8.3
150	150	12.5	14.5
210	210	11.0	13.2
270	270	5.8	3.8
330	330	3.0	3.5

Experiment 1: Sheep, R. 2; weight 36.2 kgm.; received 27.0 mgm. S per kgm.
Experiment 2: Sheep, R. 1; weight 45.3 kgm.; received 27.0 mgm. S per kgm.

However, experimental conditions are complicated by the action of the calcium salt injected previous to the magnesium sulfate on the vegetative nervous system and on tissue permeability in general; we intend to repeat these experiments with small doses of sulfates with various cations using the improved analytical method of White.

The results obtained in our experiments with phosphates differ markedly from those reported by Denis. A definite retention of phosphates was ascertained by blood analyses, though the dose employed was only one-fourth of Denis' dose; we thought, however, that no definite conclusion could be drawn from blood analyses alone; the results from the experiments where the urinary excretion of intravenously injected phosphates was followed confirm our statement as to the retention of phosphates; the excretion of intravenously injected phosphates is rapid in the first period of fifteen minutes, during which period there is a great excess of phosphates (in our experiments about 40 mgm. P per 100 cc.) in the body fluids; as soon as the phosphate concentration drops to about twice the normal level, retention becomes more evident. Since only 19 to 22 per cent of the phosphorus introduced reappears in the urine within four hundred minutes, it seems that the kidney has a comparatively low

TABLE 5

TIME AFTER INFUSION			VOLUME OF URINE SPECIMEN			CONCENTRATION RATIO		
Experiment 1	Experiment 2	Experiment 3	Experiment 1	Experiment 2	Experiment 3	Experiment 1	Experiment 2	Experiment 3
minutes	*minutes*	*minutes*	*cc.*	*cc.*	*cc.*			
30	30		60	70		6.4	5.2	
60	60	45	48	80	56	4.0	1.7	3.6
90	90	90	36	61	27	0.7	0.6	0.8
120	120		30	48		0.9	0.9	
180	180	175	55	75	43	1.2	1.0	1.7
240	240	240	50	94	50	1.5	0.9	2.3

Experiment 1; Sheep Br. 1; received a total of 0.5 gram P.
Experiment 2: Sheep, Br. 3; received a total of 0.5 gram P.
Experiment 3: Sheep, R. 1; received a total of 0.5 gram P.

permeability for the phosphate-ion; this is obvious when the rate of phosphate excretion is compared with the rate of elimination of the chloride-ion, as shown in the following.

Molar concentration of P after infusion of 1.30 grams P into a 50 kgm. sheep, (assuming the body fluids to total 3000 cc.)..... 0.0039 (1)
Molar concentration of Cl after infusion of 9.009 grams Cl into the same animal..... 0.049 (2)
t_1 period of excretion of 1.3 grams P..... 240 minutes
t_2 period of excretion of 9.09 grams Cl..... 40 minutes

We want to compare t_1 and t_2 so we take P_4 0.004 M instead of 0.0039 and Cl_2 0.05 M instead of 0.049 and multiply P_4 0.004 M and 240 minutes by 12.5
We then say:

P_4 0.05 M disappears within 3000 minutes.
Cl_2 0.05 M disappears within 40 minutes.

or:

$t_2:t_1::1:75$..... (3)

In comparing the results of the phosphate elimination experiments with those on sulfates a fundamental difference is obvious; the plasma-phosphorus concentration is highest immediately after the injection of phosphates, and shows a slow but steady decline; after the intravenous injection of sulfate a maximum is reached within one hundred and fifty minutes after which period a rather abrupt decline to the normal sulfate level occurs. This phenomenon might be due to the concurrent influence of the calcium-ion; or to the storage in a tissue other than blood and subsequent excretion of the sulfate.

CONCLUSIONS

1. Intravenously injected chlorides disappear almost instantaneously from the blood stream; the chlorine probably permeates the kidney easily and also diffuses freely into the tissue spaces, thus causing a rapid drop in the plasma-chloride concentration.

2. Intravenously injected sulfates are retained and probably stored, for they reappear only after a long period, and then disappear suddenly from the blood stream.

3. Intravenously injected phosphates are retained for a long period; excretion and diffusion is rapid as long as the high partial pressure of dissociated phosphates is maintained; but on the return to a level near the normal, disappearance from the blood stream of the phosphate-ion becomes very slow.

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CENTRAL NERVOUS EXCITATION BY ALKALOIDS IN INSECTS

W. J. CROZIER AND G. F. PILZ

From the Zoölogical Laboratory, Rutgers College

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The possibility of utilizing the selective action of drugs for purposes of functional analysis (1) in the central nervous system is increased by the finding of resemblances in the actions of neurophil substances in animals more or less closely related. Peculiarities encountered in experiments with lepidopterous larvae (2) made it desirable to extend the observations to an imaginal insect. The common red-legged grasshopper (*Melanoplus femur-rubrum*) was found convenient (3). Parallel experiments have been made with the larvae of *Tenebrio*, but for present purposes the results obtained with grasshoppers are more significant.

The drugs used were tested in two ways, namely, by injection and by direct application to the thoracic ganglia alone. In experiments of the former type the injection was made ventrally in the second abdominal segment, to one side of the mid-line, the needle pointing anteriorly and inserted immediately beneath the chitin. The volume of fluid introduced was 0.2 cc., sometimes less. With such small amounts of substance fairly concentrated solutions were employed.

The direct application of drugs to the thoracic ganglia was carried out with the grasshopper held in a clip grasping the wing covers, the chitin over the region of the ganglia and the covering membrane having been removed with a sharp scalpel. Solutions (in Ringer's fluid) were applied to the nerve cord as small drops, the grasshopper being placed dorsal surface up. The application was in this way fairly well localized to the nerve cord.

Small amounts of adrenalin, injected, led to regurgitation and convulsive movements of the palpi. Application of adrenalin to the nerve cord, even in 1:1000 concentration, was without effect. In the case of the other substances used, no material difference was encountered in the results of the two methods of administration.

General excitation was produced by strychnine (1:100), nicotine (1:1000), veratrine (one hundredth saturated), atropine (0.01 satd.), caffeine (1:200) camphor (0.01 satd.) and phenol. Picrotoxin (0.01 satd.) induced only continued twitching movements of the palpi.

When saturated or slightly weaker camphor solution was applied to the exposed ganglia evidence was obtained of inhibitory reversals. If one strokes the mouth parts of a normal grasshopper, prepared for stimulation of the thoracic ganglia, the mouth parts close upon the object and the legs attempt to seize it. After application of camphor the mouth parts still bite a needle touching them, but the legs are extended outward and upward.

Grasshoppers injected with 1:1000 nicotine also give evidence of the excitation of definite motor elements. In such animals stroking the abdomen or pinching the femur of a hind leg led to sudden outstretching of the two posterior pairs of legs; the anterior pair was not especially stimulated in this response.

Grasshoppers and caterpillars (2) agree in showing central nervous excitation by strychnine, pilocarpine, nicotine, atropine, caffeine, camphor and phenol; for the grasshopper picrotoxin was found less effective than with various caterpillars. These forms agree in showing strychnine to be relatively ineffective in excitation (concentrated solutions must be applied). There is no evidence in these insects of a synaptic effect of strychnine.

The central nervous system (cord) of the insects thus far examined corresponds with that of the crayfish (4) in being stimulated by strychnine, picrotoxin, nicotine, atropine, caffeine, camphor and phenol. These arthropods all differ from the earthworm, however, in that the neural cord of the latter (5) is insensitive to applications of nicotine, phenol and caffeine. This finding may be taken provisionally to justify the suspicion that the central nervous tissues of arthropods possess certain common features, revealed through the actions of neurophil drugs; and that these properties distinguish the arthropod central nervous system from that of an oligochaete. To what extent the cellular differentiation and the chemical constitution of the neurones involved (6) may respectively determine such differences, it is scarcely possible to say.

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TEMPERATURE EFFECTS IN THE HEARTS OF A TWINNED EMBRYO

W. J. CROZIER AND CARL L. HUBBS

From Rutgers College and the University of Michigan

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A group of embryonic teleosts, homogeneous as to origin and history, exhibits a fair degree of uniformity in the heart rates of the different individuals at a given temperature. This has been taken to signify[1] that the mass of substances (? enzymes) controlling the chemical processes underlying the heart beat[2] are in close correspondence, through inheritance, in the different individuals of such a group. From this standpoint, a study of the heart rates in twinned fish embryos may give evidence as to the degree of chemical correspondence in monozygotic twins. The structural similarities of "identical" twins are clearly indicative of a certain basic identity of compositions; few observations seems to have been made, however, as to the nature and extent of chemical parallelism in the twin products of a single ovum.[3]

Among a number of embryos of a Lake Ontario race of the lake-herring, *Leucichthys artedi*, there was noted one at the hatching stage which was quite symmetrically twinned, having two heads well separated, two hearts, and the bodies united posterior to the mid-point. The heart rates in these twins were determined over a range of temperatures. A set of readings is given in figure 1; in this figure the circlets represent single observations (reduced to beats per minute). Several points are emphasized by this graph: the close parallelism of the temperature-rate relations in the two hearts; the simultaneous cessation at a high temperature; and the simultaneous recovery on cooling (the embryo was under continuous observation). The temperature coefficient and the magnitude of the heart rate are not significantly different in the twin embryo from that found in other individuals at the same stage (cf. figs. 1, 2). And the differences between

[1] Loeb and Ewald, Biochem. Zeitschr., 1913, lviii, 179.

[2] Loeb and Chamberlain, Journ. Exper. Zoöl., 1915, xix, 559.

[3] Moore, This Journal, 1918, xlv, 188.

[3] Bateson (1913, Problems of Genetics, p. 44) cites after Nettleship two cases of "identical" human twins in which one of each pair was color-blind and the other normal. The close physiological correspondence of human identical twins is discussed by Gesell, Sci. Mon., xiv, p. 305–331 (1922).

the heart rates of the twin components, at any one temperature, is about as great as that found between two normal individuals. The hearts of a twinned Fundulus observed by Chidester[4] are stated to have had rates of beating 90 per minute and 110 per minute for the left and right components respectively (presumably at the same temperature); but in this case the left component was smaller, and had the right eye much reduced.

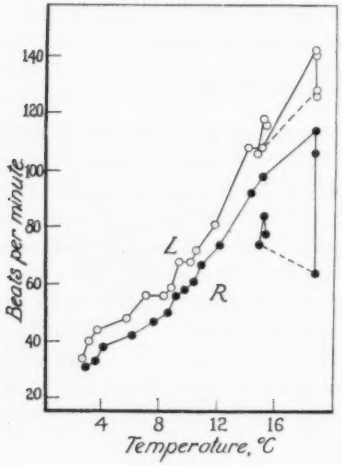

Fig. 1

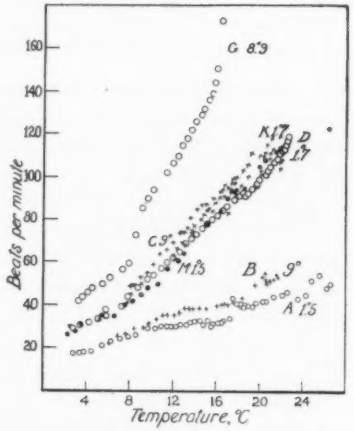

Fig. 2

Fig. 1. The rates of heart beat in a twinned embryo of Leucichthys at temperatures between 2.6° and 18.8°; *L*, left twin; *R*, right twin. Note simultaneous drop in heart rate at 18.8° and simultaneous recovery upon cooling. The lines connect successive observations. Rate of temperature increase in the experiment, 0.4° per minute. For 33 days preceding these observations, this embryo had been developing at a temperature of 8.4° to 9.4° C.

Fig. 2. Showing that the magnitude of the temperature effect upon heart-rate is a function of the degree of development, not of the temperature (indicated) at which the embryos have been developing. Curves are shown for only a few typical individuals of increasing development stage (individuals *A* to *G*). The curves *C* and *M* include between them the range of variation found at this stage of development, in embryos raised at 1.5°, 1.7°, or 9.0°. Immediately after hatching the heart rate is found much higher, *G*, than just before this event *K*, in correlation with swimming and other movements which appear after hatching.

The uniformity of heart rate tends to disappear, among a given group of embryos, as development proceeds,—owing to the entrance of secondary disturbing factors. In general, also, the rate increases with the degree of development (fig. 2). It is significant that the magnitude of the heart

[4] Chidester, Anat. Rec., 1914, viii, 367.

rate, over a range of temperatures, is a function of the stage of development, but not of the particular temperature at which the embryos have been maintained during growth (fig. 2). The temperature at which the heart of an embryo begins to exhibit irregularities and the onset of heat-stoppage, as the temperature is increased, is also independent of the temperature to which the embryo has been habitually exposed,[5] which in these experiments was 1.7° to 9° in different cases. The heart rate may thus be regarded as primarily dependent, during early growth, upon the intrinsic chemical endowment, rather than upon adaptive metabolic changes correlated with the environmental temperature.

These findings permit the comparison of the heart rate curves of our twinned embryo with the similar data for other embryos at about the same developmental stage. Such comparison makes it clear that the components of this embryo resemble one another closely, especially in the effect of heat in producing heart-stoppage. In a number of tests under comparable conditions the temperature producing heart-stoppage was found for different individuals; it ranged from 20.3° to 29.4°, in every case the upper limit for normal heart beat being higher than in the twins. Moreover, this upper limit, and the temperature at which resumption of normal beating reappeared on cooling, showed no close agreements in the different cases.

To what extent this correspondence in the twinned embryo may be due to the possession of a common circulation can hardly be decided; but it is noteworthy that the heart rates are not *identical*, but consistently differ from one another by a certain amount whatever the temperature (fig. 1) —hence a general metabolic control of the heart rate can scarcely be postulated. The conditions of parallel heart-rate curves, of identical temperature of heart-block, of simultaneous resumption of beat on cooling, and of lower maximal temperature for beat (as compared with un-twinned embryos), are better understood as resulting from a closely similar intracardiac chemical condition in the twin members, resulting from their origin on a single blastoderm.[6]

It is hoped to extend these observations,[7] which for the present are suggestive merely.

[5] According to Jacobs (Journ. Exper. Zoöl., 1919, xxvii, 427) echinoderm larvae show a quite negligible thermal adaptation.

[6] In Ambystoma embryos Laurens (1914, This Journal, xxxv, 207) has shown it improbable that nervous connections interfere with the temperature effects on the musculature of the heart.

[7] Our observations were made during the course of work by one of us (C. L. H.) with embryonic teleosts in the Bionomics Greenhouse of the University of Chicago.

STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

XIII. A Sympathetic and Adrenal Mechanism for Mobilizing Sugar in Hypoglycemia[1]

W. B. CANNON, M. A. McIVER[2] AND S. W. BLISS

From the Laboratories of Physiology in the Harvard Medical School

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When insulin in an amount which reduces blood sugar below the common physiological percentage is administered to persons suffering from diabetes, characteristic symptoms occur which have been called "hypoglycemic reactions." They include pallor, rapid pulse, dilatation of the pupils and profuse sweating (1). These are indications of activity of the sympathetic division of the autonomic system and, as is often the case when that system is excited, there are tremors in skeletal muscle. Similar signs—dilatation of the pupils, erection of the hair, salivation—have been reported as occurring in cats after insulin injections (2), and these too are explicable as results of sympathetic nervous discharge. A natural inference from this evidence that the reduction of blood sugar by insulin involves sympathetic impulses is that adrenal secretion, known to be subject to such impulses, might be increased. Stewart and Rogoff have reported that in three cats, in which the influence of insulin on adrenal secretion was investigated, "no definite effect of any moment could be made out" (3). Since their method, however, gives negative results in their hands and positive results in other hands (4), their failure should not be regarded as significant.[3]

[1] A preliminary report of this work was published in the Boston Medical and Surgical Journal, 1923 (July 26), clxxxix, 141.

[2] Medical Fellow of the National Research Council.

[3] Stewart and Rogoff continue to trust their "straightforward way" of estimating adrenal secretion (collecting blood from a "cava pocket" and assaying its adrenin content outside the body), although other methods, in the hands of different observers, have yielded concordant results quite contrary to theirs. An illuminating commentary on the reliability of their procedure is afforded by Kodama (4) who has used it carefully and in many tests. Stewart and Rogoff declare that the average output from the adrenals of the cat under their experimental conditions is between 0.00021 and 0.00025 mgm. per k. per minute (5); Cannon and Rapport (6) measured it and found approximately 0.0007 mgm. (an amount not very different from the average of 0.0006 mgm. which Stewart and Rogoff (7) had estimated previously by use

If adrenal secretion is increased as blood sugar falls after injection of insulin, the increase would indicate special activity of the sympathetic division of the autonomic system, and the two together—extra adrenin and sympathetic impulses—would have as a natural consequence a mobilization of sugar from the liver. Thus an automatic recovery of a disturbed equilibrium would be provided for. The interesting possibilities in these conjectures led us to put them to experimental test.

THE METHOD. As an indicator of adrenal secretion we have used the heart denervated by removal of both stellate ganglia and section of both vagus nerves. Sympathetic supply to the thyroid gland is thereby eliminated. Previous studies have shown that in animals with the hepatic nerves severed, the acceleration of the heart thus denervated, when an afferent nerve is stimulated or asphyxia is produced, is due solely to increased discharge of adrenin into the blood stream (6) (9), and that, if animals are fasting, the hepatic factor is so slight compared with the adrenal that it may be disregarded (10). The observations on the denervated heart of cats have been confirmed by Searles in studies on the denervated heart of dogs (11).[4]

of methods which they later repudiated); now Kodama, using the "cava pocket" method, reports the average figure as 0.00065 mgm.

Stewart and Rogoff affirm that 0.00025 mgm. is the steady unvarying secretion, not changed by afferent stimulation (8); Cannon and Rapport quantitated the secretion when increased by afferent stimulation and observed that it ranged between 0.0032 and 0.0049 mgm. per k. per minute; Kodama's assays under the same conditions, but on "cava pocket" blood, though averaging considerably less, ranged as high as 0.0047 mgm.

Rogoff, in a recent comment on Kodama's paper, declares that Kodama, "although he has evidently spent much time" on the method, has not mastered it, and that one of the best proofs that he does not know how to make the assay is the "excessive outputs obtained by him" (28). There is assumption here that Stewart and Rogoff's low figures are correct, and that Kodama's higher figures are not. The same method brought forth the two kinds of results, and therefore cannot be used to decide which is correct. Stewart and Rogoff have had no support from other observers for their constant assays and for their conclusions therefrom. The first observer to employ their method outside their laboratory obtains results which agree, quantitatively, with the results found by Cannon and his collaborators.

[4] In a recent paper (12) Stewart and Rogoff have reiterated that they have caused reflex acceleration of the denervated heart after suppression of the adrenal output. We call attention again to the proof *a*, that the residual increase of rate after adrenalectomy is due to a discharge from the liver, and *b*, that if the liver nerves have been severed the effect from the adrenals persists, but disappears as soon as these glands are removed (6). Furthermore, as Cannon and Carrasco-Formiguera showed (9), after section of the hepatic nerves the denervated heart can be reflexly accelerated when the blood flow from the adrenals is free, cannot be accelerated when the flow is blocked, and can be accelerated again when the block is removed. These two lines of evidence for reflex increase of adrenal secretion, which are irreconcilable

Our experiments, which were performed on cats, were at first done under anesthesia—a condition which may profoundly affect the metabolism of sugar in the body. Griffith (15) has proved, however, that chloralose anesthesia is satisfactory for studies of physiological factors influencing the glycemic (i.e., the blood sugar) concentration. Deep chloralose anesthesia, in our experience, though not preventing a fall of the glycemic percentage after insulin, does greatly reduce or abolish the hypoglycemic reactions. Since they are signs of disturbance in nerve centers, their absence under deep anesthesia is readily comprehensible. A satisfactory dose of chloralose for our purposes was found to be 0.1 gram per kilo by mouth. We have followed Griffith's method of administering it in milk which the animal drank, or we have given it in solution through a stomach tube (10 cc. of 1 per cent per kilo). During the short time while the chest was opened for removal of the stellate ganglia, ether was commonly administered to a degree which prevented reflex movements. Only a slight amount of ether was needed, and it was discontinued as soon as the operation was ended. The animals were well nourished and had been fed meat the previous afternoon.

Observations were made also on unanesthetized animals in which the heart had been denervated aseptically, and which were living normally in the laboratory.

The insulin (Lilly's) was injected into the jugular vein, 4 units per kilo being the usual dose. The blood for assay of its sugar concentration was taken from a carotid artery in the anesthetized and from a jugular vein in the unanesthetized animals, and was tested by the Folin-Wu method (14).

The temperature of the anesthetized animals was kept uniform within a degree centigrade throughout each experiment.

The heart rate was recorded in blood-pressure records in the observations on anesthetized animals (see fig. 5); it was counted by use of the stethoscope, for 15-second intervals, in the unanesthetized animals.

with Stewart and Rogoff's denial of that secretion, they have not mentioned, though published many months before the appearance of their paper.

The method used by Cannon and Carrasco-Formiguera to prove *reflex* control of adrenal secretion was so close a repetition of Stewart's method of proving *direct* splanchnic control that Zunz and Govaerts (13) overlooked the difference and stated that Cannon confirmed Stewart. The identity of methods and results raises the question whether the proof for direct nervous control of adrenal secretion is to be rejected or the evidence for reflex control is to be accepted.

Rogoff has just published the observation that asphyxia of bulbar centers induces an increased rate of adrenal secretion, but there is "no evidence," he states, "that asphyxia of the entire animal is capable of augmenting the output of epinephrin" (26). May we call attention again to the paper by Cannon and Carrasco-Formiguera (9) which reported observed facts contradictory to this conclusion and which Rogoff does not mention.

RESULTS. We shall report first the observations on animals under chloralose anesthesia, and thereafter, at each point, the corresponding observations on unanesthetized animals.

1. As the glycemic concentration falls after injection of insulin, it reaches a critical point, at which the rate of the denervated heart begins to be accelerated, and as the sugar percentage continues to fall the heart rate continues to rise until a maximum is reached (13 cases). This observation is illustrated in figures 1, 2 and 5. As shown in figures 1 and 2, the acceleration is usually sharp when the standard dose, stated above, is given. When a smaller dose is given (see fig. 7) the rise of rate is more gradual. The maximal increase in the anesthetized cases was 48 beats per minute, the minimum, 24. The faster rate may continue for 2 hours or more.

In unanesthetized animals the acceleration was more marked for the standard dose than in the anesthetized. For example, in the case illustrated in figure 2, the increase was as much as 80 beats per minute, and the rate continued high (more than 30 beats per minute above the basal rate of 112) for more than three hours. With smaller doses the increases were smaller—as low as 20 beats per minute. The protocol of the observations illustrated in figure 2 is as follows:

Cat 232, with heart denervated. November 30, 1923, brought from animal room; 12:00 noon, heart rate (h.r.) while quiet in lap, 112 per minute. 12:15, jugular bared under ethyl chloride, cat quiet; blood sample no. 1 taken, 129 mgm. sugar. 12:24, 10 units insulin (4 units per k.) injected subcutaneously on one side. 12:27, squatting on floor, h.r. 112. 12:40, quiet in lap, h.r. 112, resp. 13 per minute. 1:00, squatting on table, h.r. 112, resp. 16 per minute; nictitating membrane (denervated) one-fourth over eye. 1:20, squatting, h.r. 172, resp. 41 per minute, 1:30, blood sample no. 2 taken. 65 mgm. sugar. 1:40, squatting, h.r. 140. 1:50, h.r. 176. 2:00, h.r. 176. 2:10, h.r. 160. 2:20, h.r. 156 to 164, varying up and down during observation. 2:30, h.r. 164. 2:40, h.r. 172. 2:50, h.r. 160–172, varying fast and slow. 3:00, h.r. 160:172. 3:10, h.r. 148. 3:20, blood sample no. 3 taken, 34 mgm. sugar. 3:25, lying on side, pupils dilated, tail hairs lifted, h.r. varying between 176 and 192. 3:30, weak, unable to stand. 3:40, lying on side, resp. 140 per minute, pupils dilated, nictitating membrane disappeared, salivation. 3:45, h.r. 164, with frequent dropped beats. 4:00, lying on side, legs stretched out, tail curved over back, h.r. 164. 4:10, same state, occasional stretching out, very rapid breathing, h.r. 188. 4:20, same state, h.r. 140 (?) paired beats. 4:23, convulsion: blood sample no. 4 taken, 32 mgm. sugar. 4:28, glucose injected under skin left side. 4:30, h.r. 158.

2. The critical point at which the denervated heart of the animal under chloralose begins to beat faster appears to lie within a range between 110 and 70 mgm. of glucose per 100 cc. of blood. We say "appears" because in judging the matter we have to assume that the initial drop in the glycemic concentration follows a fairly straight line between the first two assays. It has been reported that after insulin injection in rabbits the blood sugar falls at a practically uniform rate (18). As

shown in figure 1B and in figure 5, the first three assays lay in a line, and in those cases we may be fairly sure of the critical range; in figure 1B the faster rate started at a level of 70 mgm. and in figure 5 at a level of about 110 mgm. Other cases (e.g., fig. 1, A and C, and fig. 7) lie within these limits.

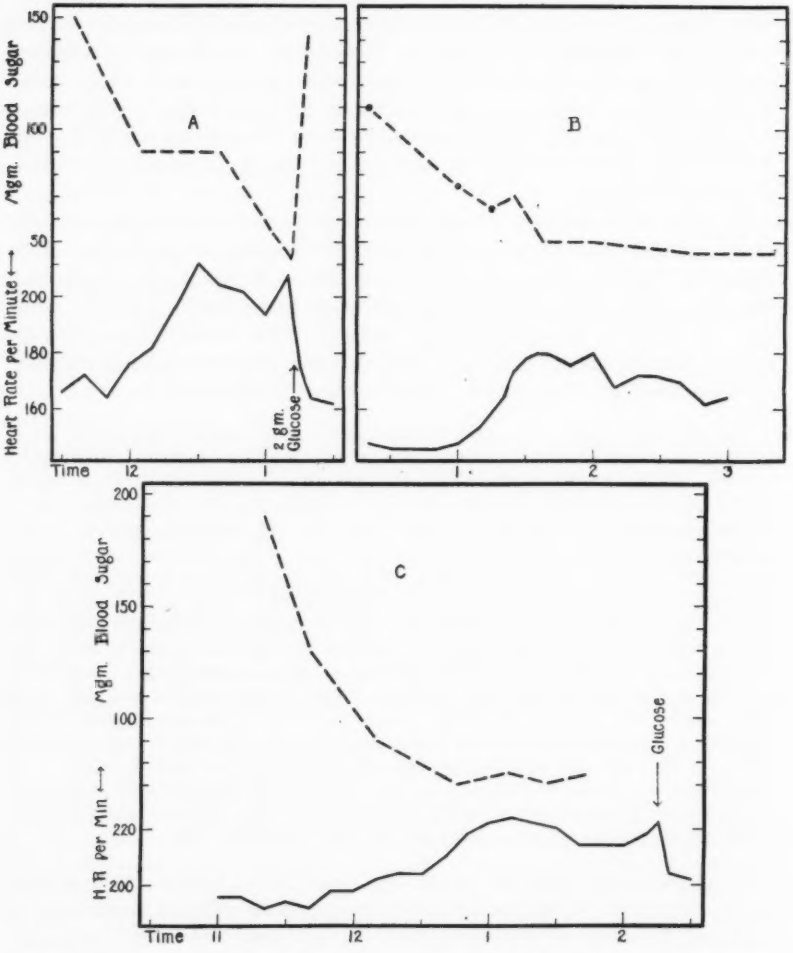

Fig. 1. Increase of rate of the denervated heart (solid line), in animals under chloralose anesthesia, when the falling blood-sugar concentration (dash line) passed a critical point. In case A, the insulin was injected into the jugular vein at 11:33; in B, at 11:08; and in C at 9:30. In each case 4 units per kilo were injected.

Figure 2 shows well the remarkable change which occurs at the critical point in the unanesthetized cat. At 1 o'clock the heart rate was 112 and the respiration 16 per minute; at 1:20 the heart rate was 172 and the respiration 41. If we may assume that the blood sugar was dropping uniformly the concentration was between 70 and 80 mgm. per 100 cc. when the heart began to speed. This coincides with clinical observation, for Fletcher and Campbell testify that "when a reaction has already been experienced the onset of a subsequent one is usually recognized by

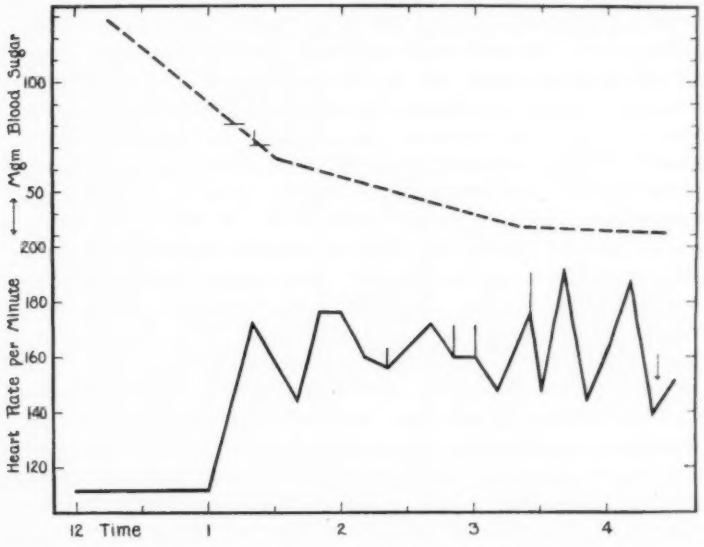

Fig. 2. Increase of the rate of the denervated heart (solid line) in an unanesthetized animal, when the falling blood-sugar percentage reached about 0.07. The rate continued high and after an hour or two was spasmodically increased still further, as represented by the height of vertical lines at several counts. A convulsive seizure (see arrow) occurred at 4:23, 4 hours after the subcutaneous insulin injection (4 units per k.), and about 3 hours after the quick rise in the heart rate.

the patient when the blood-sugar percentage falls to some point between 0.08 per cent and 0.07 per cent." And again, "when the blood sugar percentage falls to 0.07 per cent under the influence of insulin, the patient becomes aware of it" (1).

Why, in our cases, the critical level was higher as a rule in animals under chloralose than in the unanesthetized animals is not clear. One would suppose that the anesthetic would decrease rather than increase sensitiveness. If the anesthetic is given to excess, it may decrease the sensitiveness to the extent of wholly abolishing the reaction, as already

noted. Animals lightly chloralosed, however, are hypersensitive to sounds and jars, as indicated by spasmodic response to such stimuli, and they frequently jerk in an incoördinate manner. These are signs of instability in motor centers. Possibly in some instances the nerve cells responsible for the hypoglycemic phenomena are rendered hypersensitive to effects of insulin by chloralose.

In our experience the acceleration of the denervated heart appears before other striking signs of sympathetic activity. In the case illustrated in figure 2, for example, the faster rate was first noted at 1:20; for the first time at 3:25 were the pupils seen to be dilated and the hairs of the tail standing erect. Unfortunately we have no convenient means of recording early effects on the hairs; and the iris is subject to control by way of the short ciliary nerves, even though the stellate ganglia have been removed—a control which may overcome the action of an opposing factor. The absence of obvious changes in the hairs and in the iris need not be regarded, therefore, as very significant. It is possible, however, to obtain evidence of sympathetic activity in the heart itself. In two cases under chloralose the adrenal glands were removed and the vagi cut, but the stellate ganglia were left intact so that the heart could be influenced directly by sympathetic impulses. In both cases the heart accelerated when the glycemic concentration fell below 80 mgm.—in one case 10 beats and the other 16 beats per minute. These results show that the cardio-accelerator nerves themselves may be stimulated at the critical range.

3. If the adrenal glands have previously been removed, or if one has been removed and the other denervated, a fall of the glycemic percentage below the critical range is not accompanied by an increased rate of the completely denervated heart (4 cases). This observation is illustrated in figure 3. It represents graphically the conditions under chloralose in an animal from which the left adrenal gland had been removed and in which the right splanchnic and the hepatic nerves had been cut 19 days previously. The concentration of blood sugar fell to 50 mgm. with no increase in the heart beats per minute as the critical range was traversed. In other instances, levels of 40 and 44 mgm. have been reached under such conditions, or with both adrenals absent, without calling forth any noteworthy acceleration of the pulse.

In figure 4 are represented the changes in an unanesthetized cat with denervated heart and with one adrenal previously removed, and the opposite splanchnic nerves severed, occurring after intravenous injection of 4 units of insulin per kilo. This figure should be compared with figure 2. As the blood sugar fell through the critical range, and further to less than 50 mgm., the heart rate increased 6 beats per minute—an insignificant change— instead of increasing 60 or more beats per minute, as in figure 2. The protocol of the observations illustrated in figure 4 is as follows:

Cat 239, with heart denervated, and with right adrenal removed and left splanchnics cut. Weight, 3.1 k. January 11, 1924. 10:40, h.r. 144. 10:50, h.r. 144. 10:52, blood sample no. 1 taken from jugular vein under ethyl chloride, 200 mgm. sugar. 10:54, 12 units insulin in jugular. 11:00, h.r., 144. 11:10, h.r. 144. 11:20, h.r. 144. 11:26, blood sample no. 2 taken, 95 mgm. sugar. 11:30, h.r. 148. 11:40, h.r. 148, intestinal gurglings heard. 11:50, h.r. 148. 11:58, blood sample no. 3 taken, 57 mgm. sugar. 12:00, h.r. 148, hairs of back and tail lifted. 12:10, h.r. 148, hairs very fuzzy, animal restless (change from previous quiet). 12:20, h.r. 150, cat weak, staggered while walking. 12:26, blood sample no. 4 taken, 51 mgm. sugar, cat weaker, unable to stand. 12:30, lying on side. Periodic rapid panting, mouth wide open, h.r. 144. 12:33, convulsion. 12:38, blood sample no. 5 taken, 46 mgm. sugar; 10 cc. 10 per cent glucose injected under skin on one side. 12:45, h.r. 144, still panting.

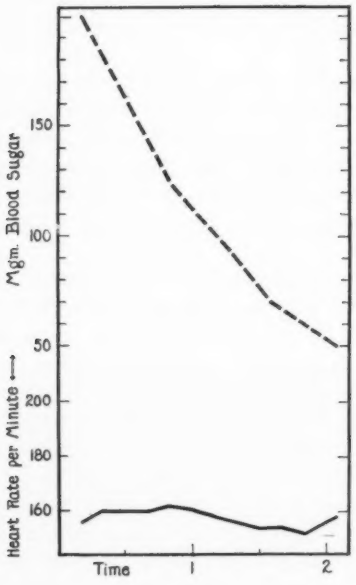

Fig. 3

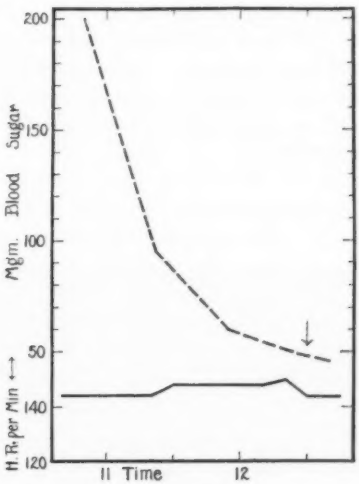

Fig. 4

Fig. 3. Failure of increase of rate of the denervated heart (solid line) in an animal under chloralose when the falling blood-sugar concentration (dash line) passed the critical range. The left adrenal had been removed and the right splanchnic and the hepatic nerves severed 19 days before. Insulin (4 units per k.) was injected intravenously at 12:19.

Fig. 4. Failure of noteworthy increase of rate of the denervated heart (solid line) in an unanesthetized animal when the falling blood-sugar concentration (dash line) passed the critical range. The left adrenal had been removed and the right splanchnic nerves severed months before. Insulin (4 units per k.) was injected intravenously at 10:54. A convulsive seizure (see arrow) occurred at 12:33, an hour and 39 minutes after the insulin injection.

These observations indicate that the cardiac acceleration reported in previous sections and shown in figures 1 and 2 is not due to direct action of insulin, or to the effects of any changes due to insulin, on the heart itself. This evidence coincides with the observations of Hepburn and Latchford on the excised heart (17). Further, neither insulin, nor any disturbance produced by it, acts directly on the adrenal gland for, as shown in figures 3 and 4, one adrenal may still be present, and, if denervated, it does not exercise any notable influence on the heart rate. Moreover, in the case represented in figure 4 as well as in other similar cases, although one adrenal gland had been removed and the other denervated, *hepatic* nerves were still existent. As the falling glycemic percentage passed the critical range, however, the rate did not become markedly faster, i.e., nothing was given off from the liver that accelerated the pulse to any considerable degree.

The conclusion which we feel justified in drawing from the combined results thus far reported is that the acceleration of the denervated heart is due to a discharge of adrenin from the adrenal glands in response to nervous impulses. In other words, these glands take part in the hypoglycemic reactions; the sympathetic discharges which cause dilatation of the pupil, acceleration of the heart, etc., also evoke increased adrenal secretion.

4. If the rate of the denervated heart has been increased because of hypoglycemia, intravenous injection of glucose promptly reduces the rate. This observation is illustrated in figure 1, A and C, and in figure 5. In the case represented in figure 5, a record was taken during the intravenous injection of 15 cc. of 5 per cent glucose, and at one-minute intervals thereafter. In 65 seconds after starting the injection the rate had fallen from 174 to 160 beats per minute, after another minute it had dropped to 144, and two minutes later it was down to 141—a rate below the original. In 20 minutes it had recovered from this depression and was beating 152 per minute. In our experience, the injection of glucose (1 gram) into an animal without adrenal secretion does not thus promptly decrease the heart rate. Since the faster rate is ascribable to increased adrenal secretion called forth by disturbed cells in the central nervous system, the restorative action of glucose can be accounted for on the assumption that it provides material which these cells need, or modifies factors which have come into play because the glucose supply has been reduced. The cells, no longer excited or rendered hyperexcitable, cease to discharge impulses via the sympathetic. Extra adrenal secretion quickly stops and the heart rate therefore quickly falls.

5. As the rate of the denervated heart increases (indicating adrenal secretion), the rate of drop in the glycemic percentage decreases, i.e., the sugar curve tends to flatten. This observation is illustrated in the

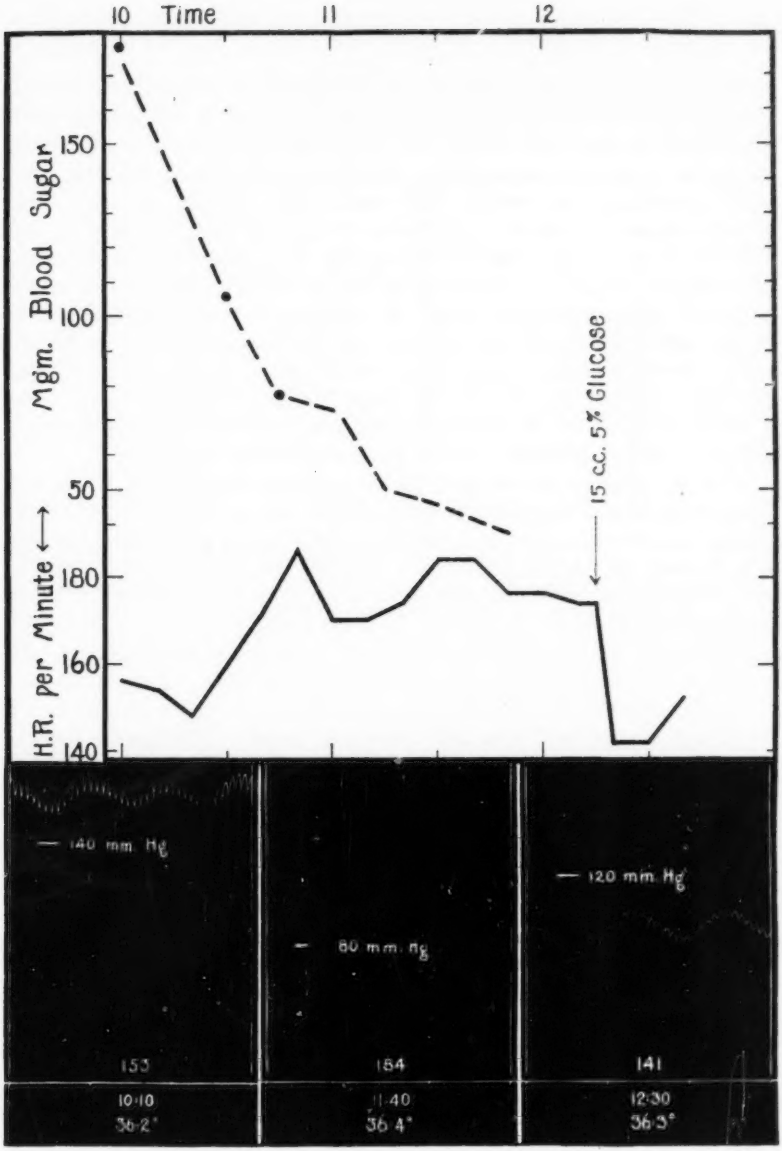

Fig. 5. Increase of rate of the denervated heart (solid line) in an animal under chloralose anesthesia, when the falling blood-sugar concentration (dash line) passed a critical point. Insulin given intravenously at 10:01. At 12:15, 15 cc. of 5 per cent glucose were injected intravenously. Portions of the original records show the method of recording the heart rate in this and in other experiments under anesthesia; the numbers above the base line (zero blood pressure; 5-second intervals) represent the heart rates per minute and the figures below, the time and rectal temperature.

three cases represented in figure 1 and in figures 2, 5 and 7. In clinical cases (27) and in laboratory experiments (16) tests of blood sugar after injection of insulin have shown that the flattening of the curve, following an initial drop, is a characteristic feature. As shown in figure 1C, the sugar percentage may remain fairly constant at a given level; or it may remain constant or nearly constant for a time and later fall, as in figures 1A and 1B; or it may continue falling but at a slower rate than before, as in figures 2 and 5. These variations in the blood-sugar curves are probably to be explained chiefly by differences in the glycogen stores in the different animals, for Macleod and his co-workers have published curves showing similar variations in rabbits that were glycogen-rich and glycogen-poor (16, fig. 6). In our cases, however, the check in the rate of fall in the glycemic concentration was observed when adrenal secretion occurred, as was manifested by an accelerated heart beat.

6. If the adrenal glands have previously been removed, or if one has been removed and the other denervated, the rate of drop in the glycemic percentage after the standard dose in animals under chloralose is usually not checked at the critical level; it may be retarded slightly or not at all at that point, or it may flatten at a very low concentration. These observations are shown graphically in figure 3 and in figure 6, A, B and C. An exception to the general statement is illustrated in figure 6D. In that case the curve flattened between 70 and 80 mgm., although the adrenal glands were absent. The heart rate, however, had been unaccountably high (never less than 254 beats per minute) throughout the experiment, the blood pressure when the curve flattened was only 70 mm. Hg, and the animal became so asphyxiated that artificial respiration had to be started. It is quite possible that in this case, as well as in others (cf. fig. 3, with hepatic nerves cut) aspyhxia played a rôle in setting free sugar from the liver, for we have noted not infrequently on taking the *late* samples in the course of an experiment that the arterial blood was surprisingly venous. The asphyxial condition might act either by stimulation of the hepatic cells from the sympathetic nerve centers (tail hairs were erect in 6D at 12:30) or by direct action on the hepatic cells.

The conditions in unanesthetized animals with inactivated adrenals but with hepatic innervation, when insulin has lowered the blood sugar, will be considered later.

7. If not too much insulin has been given, the increased rate of the denervated heart may be followed by an increase of the glycemic percentage, and an attendant fall in the heart rate. This reaction is shown in the record reproduced in figure 7. It has often been observed that the decrease of blood sugar due to insulin is followed fairly promptly, or after remaining at a low level for a time, by a rise to normal (16). We have frequently had occasion to note the reciprocals of such changes

in counting the rate of the denervated heart in unanesthetized animals which had been given sub-convulsive doses of insulin. The rate has continued uniformly for a varying time—from 50 to 105 minutes after

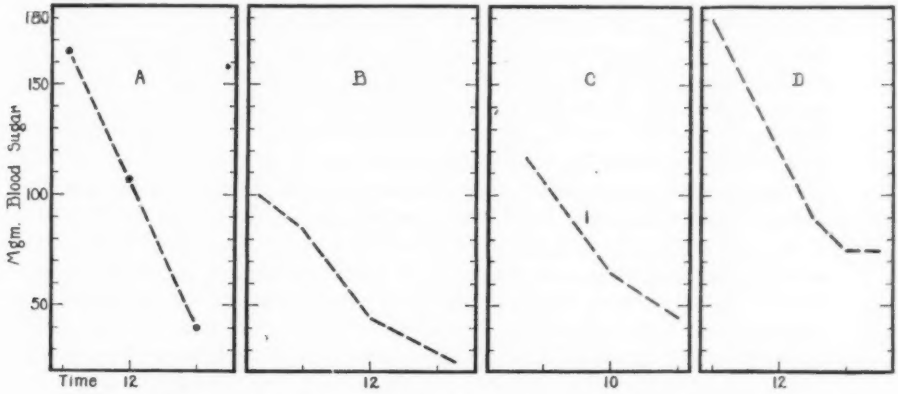

Fig. 6. The rate of decline of the blood-sugar concentration in chloralosed animals, which had been deprived of the adrenal glands. In case A the insulin (4 units per k.) was injected intravenously at 11:36, in B, at 11:06, in C, at 9:27; and in D, at 11:36. There was no check in the decline except in D (for explanation see text).

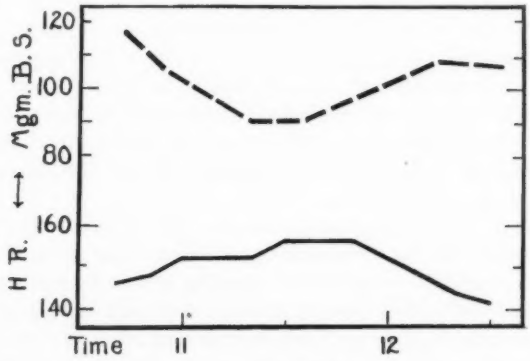

Fig. 7. Increase of rate of the denervated heart (solid line) in an animal under chloralose, as the blood-sugar concentration (dash line) fell below the critical level, and decrease of the rate as the concentration rose. One unit of insulin was given intravenously at 9:53.

the injection—whereupon it has gradually increased between 20 and 36 beats per minute, as in figure 7, and then gradually subsided again to its former level. The increased rate has not lasted longer than 120

minutes, and in one instance was over in 60 minutes. The drop in the heart rate as the blood sugar increases is just what would be expected from the observation, already noted, that introduction of glucose into the blood stream promptly brings the pulse down (see p. 54).

THE RÔLE OF ADRENAL SECRETION IN THE HYPOGLYCEMIA FROM INSULIN. The considerations presented in the foregoing pages raise the question as to the part taken by adrenal secretion in resisting the fall of blood sugar. The evidence in hand clearly indicates that this resistance and the recovery from insulin injections depend largely on the glycogen available in the liver (16). Glycogenolysis can be produced, as is well known, by direct stimulation of the hepatic nerves. Also, as Griffith has shown, it can occur reflexly in consequence of afferent stimulation, but if the adrenal glands have been excluded from action the glycemic percentage averages lower and the effect of reflex stimulation averages less than in normal animals (15). Furthermore, Trendelenburg has presented evidence that such outpouring of adrenal secretion as can readily occur in physiological states is quite capable of causing a mobilization of sugar (18). When in the early stage of an experiment the denervated heart becomes accelerated, the faster rate indicates not only that adrenal secretion is being augmented, but also by that fact that splanchnic impulses are being discharged. It is altogether probable that the liver receives these impulses just as the adrenals do. The liver, however, would be subject to both nervous and humoral stimuli—the nervous impulses and the secreted adrenin coöperating to influence it. Under chloralose anesthesia the humoral factor seems to be a necessary complement of the nervous, for, as comparison of figures 1 and 6 indicates, the course of the descent of the glycemic concentration is as a rule strikingly different, after standard doses of insulin, according to whether adrenal activity is present or absent. The character of this difference has already been pointed out (pp. 54, 56).

In the unanesthetized animal, likewise, the secreted adrenin appears to be important for the process of mobilizing sugar from the liver. In table 1 are presented the results of injecting insulin into healthy animals, with and without active adrenal glands, that had been kept under the same conditions of feeding and temperature and freedom from disturbance. (In our experience with a few cats which had just previously been subjected to prolonged excitement or which were suffering from infection, they were more sensitive to insulin than are serene and healthy animals.) In a number of cases injections were repeated in the same animal. Rabbits seem to become less sensitive to insulin as doses are repeated (25). In cats with intact adrenals this possibility was met by a larger dose at the second injection. In cats with adrenals inactivated a second dose equal to the first should have been less likely to produce convulsions,

if a sort of immunity develops. This result was perhaps indicated in cats 107 and 112, and in other cases in which the convulsive seizure occurred after a longer delay in the second than in the first test. The seizures occurred, nevertheless, and strikingly differentiated this group from the other. Among animals with normally innervated adrenals doses of insulin varying from 2 to 3 units per kilo caused convulsions in only one instance. This was in cat 228 which had had a major operation 10 days previously, and had not fully recovered from it. A dose of 2.5

TABLE 1

Presence or absence of convulsions after subcutaneous injections of insulin into (A) cats with normally innervated adrenals, and (B) cats with one adrenal removed and the other denervated

A. ADRENALS BOTH INNERVATED				B. ADRENALS, 1 OUT, OTHER DENERVATED			
Date	Cat	Insulin units per kilo	Convulsion	Date	Cat	Insulin units per kilo	Convulsion
Nov. 10	228	2.5	After 3 hrs. 30 min.	Jan. 31	239	2	After 1 hr. 30 min.
Nov. 28	228	3	0	Feb. 8	239	1	After 1 hr. 55 min.
Jan. 9	228	3	0	Feb. 2	107	2	After 1 hr. 10 min.
Nov. 28	227	2	0	Feb. 13	107	2	After 1 hr. 23 min.
Feb. 8	227	3	0	Mar. 1	107*	2	0
Nov. 17	229	2.5	0	Feb. 2	235	2	0
Jan. 7	107	2	0	Feb. 8	235	3	After 1 hr. 32 min.
Feb. 8	245	3	0	Feb. 21	90	2	After 1 hr. 40 min.
Feb. 19	Bl	2.5	0	Mar. 1	90	2	After 1 hr. 55 min.
Feb. 21	W	2	0	Feb. 21	110	2	After 1 hr. 7 min.
Feb. 21	Y	2	0	Mar. 1	110	2	After 1 hr. 30 min.
Mar. 1	B-W	2	0	Feb. 21	112	2	After 1 hr. 35 min.
Mar. 1	G-W	2	0	Mar. 1	112†	2	0
Mar. 1	G-T	2	0	Feb. 21	116	2	After 1 hr. 18 min.
Mar. 1	M	2	0	Mar. 1	116	2	After 2 hrs. 8 min.

* Panting, weakness and vomiting after 1 hour and 24 minutes.

† Salivation, mewing, weakness and muscular twitching, beginning after 1 hour and 25 minutes, and continuing for about a half hour.

units per kilo brought on a convulsive seizure after three and a half hours; later, after complete recovery, this animal twice withstood doses of 3 units per kilo without any such disturbance. On the other hand, among animals with one adrenal removed and the other denervated, insulin doses lying between 1 and 2 units per kilo induced convulsions within two hours and ten minutes in all but three cases. In the first of these (cat 235) a dose of 3 units was later effective. In the other two cases the premonitory signs appeared, but the animals passed the crisis without a seizure. The liver

was still innervated, it should be remembered, and therefore some protection against hypoglycemia still existed. It should be noted that a dose of 2 units per kilo had no convulsive effect in cat 107 when the adrenal nerve supply was intact—indeed the only influence was an increased rate of the denervated heart by 24 beats per minute for about sixty minutes during an observation lasting three and a half hours; later, after one adrenal gland had been removed and the other denervated and the animal had recovered and was well nourished, 2 units of insulin per kilo twice produced a convulsion, in seventy and in eighty-three minutes respectively, and on a third trial caused panting and vomiting in eighty-four minutes. Comparison of figures 2 and 4 brings out similar testimony. In each case 4 units of insulin per kilo was the dose. In each case the nerves to the liver were intact, but the case of figure 4 differed from that of figure 2 in having only one adrenal and that denervated. In the animal with active adrenal glands the glycemic percentage fell more slowly, the descent began to flatten just below 70 mgm., and the convulsion appeared much later, as compared with the animal lacking active glands.

The only difference between the animals in the two groups of table 1 was presence of active adrenals in one and absence in the other. All the numbered animals had undergone a major operation (denervation of the heart), and all were living together, eating the same food and manifesting the same good health. It could be objected, nevertheless, that inactivation of the adrenals might lower the glycogen storage and therefore, in spite of other similarities, the animals without adrenals might be more sensitive to the action of insulin. According to Stewart and Rogoff, however, the formation and storing of glycogen in the liver in cats is not affected by removal of one adrenal and section of the nerves of the other (19).

From observations on rabbits which had had adrenals removed (for intervals varying from 28 days to more than 8 months) Stewart and Rogoff concluded that the action of insulin does not differ in animals with and without these glands (20). They report tests on only three adrenalectomized animals: one was given 2.7 units, a second 8.8 units of insulin per kilo and a third was given a crude preparation which caused strange and unaccountable symptoms. Only one control was reported. Opposed to this slight and frail support for a rather large inference are the results reported by Lewis (21). He made tests on 27 normal rats and on 11 rats without adrenals, that had received the same food before and since the operation, 12 days earlier. The fatal dose of the insulin he used proved to be 10 mgm. per 100 grams for the normal, and 1 mgm. per 100 grams for adrenalectomized animals. A dose of 2 mgm. per 100 grams, twice the lethal amount for the latter group, did not even produce the typical hypoglycemic symptoms in the normal animals. Similar testimony has come from Sundberg, who studied the effects of insulin on normal rabbits and

on rabbits deprived of the adrenal medulla (29). He found that a given dose caused a larger reduction of blood sugar and a more certain appearance of nervous symptoms (asthenia and convulsions) in the animals without medulla than in the normal controls. It is clear that all the evidence which we have presented above as to the importance of adrenal coöperation in protecting the body against the dangers of a too great hypoglycemia is in harmony with the results reported by Lewis and by Sundberg and offers no support for the view expressed by Stewart and Rogoff.[5]

Discussion. The typical symptoms seen after an overdose of insulin are probably due almost entirely, if not entirely, to depriving the tissues of a necessary supply of sugar. That they are not dependent on the action of insulin as such is proved by their occurrence in hypoglycemic states produced by other means. About ten years ago Fischler and his collaborators called attention to a group of symptoms—excitement, convulsions, collapse and coma—accompanying the low blood sugar produced by phlorhizin, and Fischler designated the condition as "glycoprival intoxication" (22). More recently Mann and Magath have reported that the progressive decrease of blood sugar resulting from removal of the liver is attended by characteristic symptoms, prominent among which are, first, muscular weakness and, later, exaggerated reflexes, twitchings and convulsions (23). The very rapid soothing of the disturbed nerve cells by intravenous injections of glucose, whether the disturbance has been occasioned by phlorhizin, by removal of the liver, or by insulin, points to a sugar shortage as its cause.

The deficiency of sugar is probably local, and is not necessarily a consequence of a low concentration in the blood. A number of observers have called attention to a hypoglycemia which follows an abundant ingestion of sugar (24). Folin and Berglund have noted a glycemic concentration as low as 54 mgm. per 100 cc. of blood shortly after the ingestion of glucose. This low level, which is considerably under the critical range described above, they explain by assuming that because the local stores are well filled there is no need for sugar to be in transport, and consequently the blood as a carrier is lightly loaded. It seems probable that the

[5] In an article which appeared while the present paper was in galley proof, Stewart (Physiological Reviews, 1924, iv, 183) remarks on the "flimsy foundation" of Zuelzer's theory that "epinephrin is the physiological antagonist of the pancreatic hormone and that when the pancreas is removed diabetes follows owing to the unchecked action of epinephrin given off from the adrenals." He later states that "Cannon, McIver and Bliss have put forward the hypothesis, which may be considered as an elaboration of the theory of Zuelzer, that when the blood sugar tends to fall the output of epinephrin is stimulated." Readers of the present paper are in a position to judge whether our evidence is properly described as an "hypothesis," and whether in any sense it can be correctly considered as an elaboration of Zuelzer's theory.

condition of hypoglycemia after insulin is quite the opposite. The effect of insulin is to increase the utilization of sugar by active tissues; we may reasonably suppose that the local supply is the first to be reduced; the sugar in the blood probably soon passes into the tissues depleted of their sugar stores until what may be called the sugar pressure in the blood is so greatly diminished that it no longer meets the demands. In these circumstances the hypoglycemia would be caused, not by an oversupply in the local stores, but by an undersupply and an excessive demand in them. The main source of the needed material is in the liver. If the sugar is not called forth promptly from that reserve and conveyed by the blood, the organism may be in serious peril.

The observations detailed in the foregoing pages have revealed a mechanism, or set of mechanisms, having the function of maintaining the physiological percentage of blood sugar when there is danger of deficiency. If that percentage falls below a critical point in consequence of deficiency, splanchnic neurones of the sympathetic system are set in action, as indicated by increased adrenal secretion. This is a reaction which, as seen in figure 2, may occur hours before a convulsion occurs, or may occur without a subsequent convulsion (see fig. 7). It coincides with the appearance of subjective feelings of anxiety reported by patients, and shortly thereafter with objective signs of increased sympathetic innervation. Both the nerve impulses and the secreted adrenin have the effect of liberating sugar from the liver into the circulation, and thus tending to restore the disturbed equilibrium. This may be regarded as a first line of defense against a falling glycemic concentration.

Whether this first defense is effectual or not will depend in part on the reserves which can be called upon. Macleod and his collaborators have pointed out that in fasting animals with a meager glycogen store a given dose of insulin produces convulsions more frequently and earlier than in well-fed animals with an abundance of this material (16). Since adrenin is an important factor in the humoral and nervous coöperation which influences the liver, however, it is quite possible that a low adrenin content may be the occasion of weakness in the first defence. In one of our animals, already noticed on p. 59, which received a dose of 2.5 units of insulin before it had fully recovered from a major operation, the denervated heart began to beat faster within sixty minutes after the injection and eighteen minutes later it had increased 62 beats per minute; two hours later still the rate had slowly fallen to the original level, indicating that the adrenal glands had ceased to be especially active. One might suppose that this implied that the call for sugar had been answered; but a very bushy tail revealed great activity of sympathetic neurones, and the animal, weak and panting, had a convulsion a few minutes thereafter. There may have been, of course, a using up of the glycogen reserve in this case, as well as

the adrenin reserve, but if so, the two must have run down simultaneously. On the other hand, in the case illustrated in figure 2, adrenal secretion was still being poured forth in extra amount, as shown by the accelerated heart rate, when the convulsion occurred. It is clear, then, that there may be a separation of the adrenal and hepatic factors. Unfortunately it is difficult in any case to discriminate between the two, for the conditions which might lead to depletion of the adrenal medulla would be likely to lessen the glycogen content of the liver. The possibility must be kept in mind, however, that variation in the amount of available adrenin may affect the efficiency of the first defense.

If the first defense fails to prevent the glycemic concentration from falling, there is evidence of more widespread and more effective sympathetic discharges, characterized in cats by dilatation of the pupils, erection of hairs and salivation. As shown in figure 2, this stage is associated with spasmodically increased adrenal secretion (manifested by the marked temporary increases of the rate of the denervated heart). The culmination of this stage of agitation is the convulsive attack. Such an attack is accompanied by increased adrenal discharge (in one of our cases the denervated heart increased 33 beats per minute during the muscular spasms), and it is the occasion for further liberation of sugar into the circulation (25, p. 43). In well-fed rabbits a convulsion may be followed by temporary or permanent recovery without injection of glucose (16). It is probable, therefore, that the convulsion, and the agitation immediately preceding and attending it, form a secondary defence against the damage from an insufficient glucose supply for the needy tissues.

Still another factor which may be at work, though we have little evidence regarding the rôle it may play, is the asphyxial state (i.e., the venosity) of even arterial blood in the later stages of insulin hypoglycemia. It is quite conceivable that asphyxia, which can in itself produce powerful convulsions, either coöperates at some stage with lack of glucose, or with changes induced thereby, to excite activity in the nerve cells. This possibility has been suggested also by Olmsted and Logan (2).

The setting into operation of the sympathetic and adrenal mechanism for mobilizing sugar from the liver explains a number of conditions which have been observed in man. As already noted (p. 51), when the mechanism is started by a fall of the glycemic percentage to approximately 0.07, a patient becomes aware of a feeling of nervousness or tremulousness, or weakness, or of a sense of "goneness." These are subjective symptoms which have been described as occurring when adrenalin is injected subcutaneously (26). "The reaction may go no further than this of its own accord", so Fletcher and Campbell testify (1); this relief is precisely what would occur when the mechanism proves effective. "Or it may be cut short at this stage by the administration of carbohydrate;" the increase of

available carbohydrate (sugar) is the result toward which the mechanism is operating, and which, when it is achieved, automatically cuts short the reaction. If the initial symptoms become worse, they are characterized by pallor and flushing, rapid pulse, dilated pupils and sweating, with experience of anxiety, excitement or vague emotional disturbance; again these are the severer symptoms reported by peculiarly sensitive persons when given an injection of adrenalin (26). In this connection it will be recalled that as the blood sugar falls below the critical point the denervated heart beats with increasing frequency, thus indicating both an increased adrenal secretion and a greater discharge of sympathetic nerve impulses. This relationship between hypoglycemia and adrenal output was surmised by Wilder and Boothby and their collaborators. Because of a sharp change in the rate of decrease of the glycemic percentage in a diabetic patient when the first subjective symptoms of hypoglycemia appeared, they suggested that there might be a spontaneous outpouring of adrenin at that point—a result which would tend to protect against a further decrease. And in a later paper the more rapid metabolism which set in at that point was mentioned as supporting the possibility that adrenal secretion was then called forth (26). These interesting conjectures Wilder and Boothby did not pursue further, and they have brought no evidence to support their view. The experiments presented in the foregoing pages, however, definitely confirm their insight. The use of adrenin in the treatment of insulin hypoglycemia (1) is evidently a physiological procedure; the injected adrenin is added to that normally secreted and thereby augments its efficacy, or if the adrenal medulla is more or less exhausted the injected extract has a natural replacement value. A store of glycogen in the liver is, of course, presumed.

The mechanism described in the foregoing pages acts like many others, already known, that assure stability of the organism. When the equilibrium, which normally indicates a concentration of sugar in the blood sufficient to supply needy tissues, is disturbed by serious lessening of the concentration, this compensatory mechanism is set in action to restore the equilibrium. Sugar is set free from the reserves. If the reserve station is absent, as in the experiments on liver extirpation by Mann and Magath, or if the agency reducing the sugar content of the blood is too potent, as when excessive doses of insulin are given, the mechanism is overwhelmed and the result is disastrous to the organism—convulsions and coma are followed by death. From such consequences of an inadequate sugar supply to the tissues the organism is protected by the combined activity of the sympathetic system and adrenal secretion. From the evidence adduced by Griffith, cited above, it is probable that this mechanism is normally at work well within the danger line, sustaining a requisite sugar concentration in the blood.

SUMMARY

The hypoglycemic reactions occurring when an excessive dose of insulin is given include pallor, rapid pulse, dilatation of the pupils and profuse sweating, which are indications of discharge of sympathetic impulses. The question whether adrenal secretion is involved was tested by means of the denervated heart in animals anesthetized with chloralose and in unanesthetized animals.

As the glycemic concentration falls after injection of insulin it reaches a critical point at which the rate of the denervated heart begins to be accelerated, and as the sugar percentage continues to fall the heart rate continues to rise until a maximum is reached (see figs. 1, 2 and 5).

The critical point at which the denervated heart of the animal under chloralose begins to beat faster appears to lie between 110 and 70 mgm. of glucose per 100 cc. of blood. In the unanesthetized the point lies between 70 and 80 mgm. The cardiac acceleration appears before other striking signs of sympathetic activity.

If the adrenal glands have previously been removed, or if one has been removed and the other denervated, a fall of the glycemic percentage below the critical range is not accompanied by an increased heart rate (see figs. 3 and 4). The cardiac acceleration, therefore, is not due to direct action of insulin on the heart or on the adrenal gland, but is due to increased adrenal discharge in response to nervous impulses.

If the rate of the denervated heart has been increased because of hypoglycemia, intravenous injection of glucose promptly reduces the rate (see figs. 1, A and C, and fig. 5).

As the rate of the denervated heart increases (indicating adrenal secretion), the rate of drop in the glycemic percentage decreases, i.e., the sugar curve tends to flatten (see figs. 1, 2, 5 and 7).

If the adrenal glands have previously been removed, or if one has been removed and the other denervated, and the animals are under chloralose anesthesia, the rate of drop in the glycemic percentage is usually not checked at the critical level (see figs. 3 and 6A, 6B and 6C). In unanesthetized animals without active adrenal glands the fall of blood sugar is less retarded at the critical level, and the convulsive seizures are induced sooner and with smaller doses than in animals with active glands (see table 1 and cf. figs. 2 and 4).

If not too much insulin has been given the increased rate of the denervated heart may be followed by an increase of the glycemic percentage and an attendant fall in heart rate (see fig. 7).

It is pointed out that the mechanism protecting the body from dangerous hypoglycemia probably operates in two stages—a primary stage in which sympathetic activity with adrenal secretion occurs and mobilizes sugar

from the liver; and, if this proves to be inadequate, a secondary stage in which the activities of the first stage are intensified and augmented in convulsive seizures.

The mechanism here described is another remarkable example of automatic adjustments within the organism when there is a disturbance endangering its equilibrium.

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BIOLOGICAL FOOD TESTS

VII. The Vitamin A and B Content of Fresh and Dehydrated Pumpkin

AGNES FAY MORGAN and LILLIAS D. FRANCIS

From the Laboratory of Household Science, the University of California, Berkeley

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In the course of our attempts at measurement of the amount of destruction of vitamins A and B under the influence of heating, oxidation and hydrolysis incident to various cooking processes, we have used cooked or preserved foods about which we could obtain accurate information. We were offered by the makers[1] a quantity of dehydrated pumpkin flour and specimens of the fresh pumpkins from which the flour was made along with an excellent account of the process of dehydration used. It seemed worth while to investigate the vitamin content of these food products, particularly since there seemed to be no record of any previous study of the vitamin value of pumpkins, and since the pigmented character of this vegetable made vitamin A studies upon it of particular interest. Determinations were therefore made upon the vitamin B content of the fresh and dehydrated pumpkin using mice as the experimental animal, and upon the vitamin A and vitamin B content of the dehydrated pumpkin, using rats.

The effect of dehydration on the vitamin B of pumpkin. Little is yet known of the effect of various methods of dehydration upon the vitamin B content of foods of differing types. In an earlier paper Morgan and Stephenson(1) have referred to reports of the effects of dry heat upon the stability of vitamin B, and have shown destruction of vitamin B in the globe artichoke dried at 70°C. Yet Shorten and Ray (2) have indicated the stability of vitamin B in several vegetables dried in the sun. Osborne, Wakeman and Ferry (3) have shown that yeast dried in a current of air at 60°C. suffered no loss of vitamin B. The method of desiccation as well as the character of the food dried seems to affect the potency of vitamin B in dried foods.

The stability of vitamin C to drying processes shows variation due to method, as is well illustrated by the work on milk reported by Hess and Unger (4), Hart, Steenbock and Smith (5) and Hart, Steenbock and Ellis

[1] The pumpkin flour and some of the fresh pumpkin used in this investigation were kindly donated by the Caladero Products Company of Atascadero, California.

(6), while variation in the potency among different foods has been shown by Shorten and Ray (2), Givens and Cohen (7), Givens and McClugage (8) and many others. Though it is now appreciated that vitamin B is more stable than vitamin C, the work so far reported seems to indicate variation in the potency of vitamin B, due to methods of drying and to character of food, as is the case with vitamin C.

It is some time since Funk (9) called our attention to the paucity of information about effects of dehydration on vitamin contents of foods, yet there have been few contributions to the subject made since that time. The study of the effect of a specific method of dehydration on the vitamin B content of pumpkin was undertaken to gain information on the vitamin B contained therein, which seems not to have been reported, as well as to study the effect on vitamin B of a specific method of drying.

Preparation and composition of pumpkin: The pumpkin flour used in this investigation was made from the following varieties of pumpkin:[2] Kentucky Field, Morse Marrow, Golden Kutshaw, Boston Marrow. This mixture was first dried and then ground into flour as needed. "The pumpkin in the drying period enters the drying chamber at a temperature of 115° F. (46° C.), moving forward as it dries to a maximum temperature of 160°F. (71°C.). Time during this period of dehydration is from sixteen to eighteen hours." There are "27,000 cubic feet of air per minute moving through our drying chambers while dehydrating." The manufacturers state that their pumpkin flour is dried ground pumpkin to which nothing has been added.

Analysis of the pumpkin flour showed the following proximate composition:

	Per cent
Protein	8.20
Fat	2.40
Ash	7.01
Water	5.60
Carbohydrate	76.79

Several analyses of the fresh pumpkins were made when they were first cut and later while still in use. These gave an average of 7 per cent total solids. The pumpkins were kept in a refrigerator and thus remained in good condition for about ten days.

Feeding technique: The mice used in this investigation were of the same strain as those previously described by Morgan, Newbecker and Bridge (10). The comparison as shown in table 1 between the gain in weight of our controls and the gains reported by Robertson (11) for their progenitors, fed a mixed diet, has shown that our controls made very satisfactory gains in weight, even above the averages given by Robertson.

[2] Communication from the Caladero Products Company.

TABLE 1

Gain in weight of mice fed pumpkin as source of vitamin B

MOUSE	AGE AT BEGINNING OF EXPERIMENT	DURATION OF EXPERIMENT	AVERAGE DAILY INTAKE		INITIAL WEIGHT	FINAL WEIGHT	DIFFERENCE BETWEEN EXPERIMENTAL AND CONTROL MICE*
			Vitamin B	Total food			Per cent of gain or loss
	days	*days*	*grams*	*grams*	*grams*	*grams*	
			Pumpkin flour				
70 ♂	28	43	0.3	2.2	11.5	12.5	−92
12 ♀	35	60	0.3	2.6	7.3	16.1	+15
B15 ♂		0	0.3	1.9	10.4	17.6	−51
14 ♀	35	60	0.3	2.5	8.6	16.6	+3
11 ♂	35	60	0.3	3.3	11.0	20.6	−10
187 ♂	50	42	0.3	2.6	11.7	17.0	+7
192 ♂	50	60	0.3	2.4	10.8	19.7	+33
71 ♂	28	72	0.4	2.9	9.7	23.9	+7
12 ♀	95	14	0.4	3.4	16.1	17.0	+40
52 ♀	85	31	0.4	2.0	8.2	17.3	+92
69 ♂	28	72	0.4	3.3	11.3	25.0	−10
11 ♂	96	14	0.4	2.7	20.6	21.3	−41
70 ♂	71	31	0.4	2.0	12.5	15.8	+45
B14 ♀	28	67	0.4	2.6	10.1	17.8	−27
42 ♀	81	31	0.5	2.4	10.8	20.0	+86
10 ♂	28	81	0.5	3.1	8.3	22.7	−11
B15 ♂	95	14	0.6	2.7	17.6	21.7	+70
B14 ♀	95	14	0.6	2.7	17.8	21.1	+84
			Fresh pumpkin				
44 ♂	42	76	0.3	3.1	13.9	17.1	−62
54 ♂	42	76	0.3	3.3	13.5	19.6	−28
B4 ♂	34	54	0.3	2.5	7.3	12.0	−53
6 ♂	28	32	0.3	3.1	9.3	15.3	−94
5 ♀	26	32	0.4	3.2	7.8	18.1	+14
55 ♀	80	36	0.4	3.2	13.5	21.5	+91
67 ♀	80	36	0.4	3.2	12.9	18.3	+87
B1 ♂	34	33	0.4	2.8	9.5	20.0	+14
Control averages compared with Robertson's averages*							
Control, average four mice ♀	26	84	yeast 0.1	2.6	8.7	20.6	+10
Robertson's average ♀	26	84			9.5	20.3	
Control, average four mice ♂	28	82	yeast 0.1	3.4	9.2	25.5	+32
Robertson's average ♂	28	82			12.3	24.6	

* Comparisons between mice of same sex, age, and for same experimental period.

The composition of the basal diet used in this laboratory has been described by Morgan (12). The control mice received 0.1 gram dry brewery yeast daily and the experimental mice, pumpkin flour or fresh pumpkin instead. The intake of pumpkin, both dry and fresh, is reported in terms of total solids.

To induce the mice to eat the pumpkin flour, it was necessary to mix it with a small known amount of basal diet. This mixture was placed daily on top of the basal ration or fed separately. The fresh pumpkin, ground finely, was readily eaten alone by several mice, the others were induced to eat it by diluting it with basal diet as was done with the pumpkin flour.

The curative method of feeding had to be abandoned because the mice, suffering from absence of vitamin B, could not be induced to eat the pumpkin flour.

The comparison between each experimental mouse and control mice is made from an average of four controls of the same sex, beginning at the same age and running for the same length of time as the experimental mouse (See table 1.)

Five groups of four to eight mice each were fed differing amounts of pumpkin flour or fresh pumpkin, as the sole source of the growth-promoting vitamin. Any uneaten portions were carefully secured and the weights recorded. The gain in weight stated in the table is thus relative to the amount of pumpkin consumed.

Vitamin B in pumpkin flour. The groups which ate only 0.1 gram and 0.2 gram of pumpkin flour or of fresh pumpkin daily, made gains in weight notably below that of the average of the four controls, and are therefore not reported in the table. A few growth curves from this group are given in chart 1. Those for mice 46 and 52 show clearly how inadequate 0.1 gram of pumpkin flour intake was in stimulating growth. Yeast was given to mouse 52 as indicated. The resulting stimulus to growth, following a single dose of yeast, bears a striking resemblance to the improved curve in dicating the gain in weight attained by an increase in the pumpkin flour consumed to an amount averaging 0.4 gram daily.

Mice which consumed 0.3 gram of pumpkin flour daily show gains which vary widely from one another and from the gains of the control averages. Mouse 70, started at an early age, made almost no gain, and mouse B15 a gain of only about one-half that made by its control average. The greatest gain, 33 per cent, was made by mouse 192, which had failed on a lower pumpkin flour intake.

It appears that 0.3 gram pumpkin flour eaten daily as the sole source of vitamin B is adequate for some and notably inadequate for other mice.

In the group which consumed an average of 0.4 gram daily, two mice, 69 and B14, which were started young and maintained this intake for a long period of time, each made gains below that of the control averages, but con-

siderably better gains than mice 70 and and B15, which failed so conspicuously on 0.3 gram. Mouse 71, however, started at the same age, made a gain slightly above that of the control average. The large gains

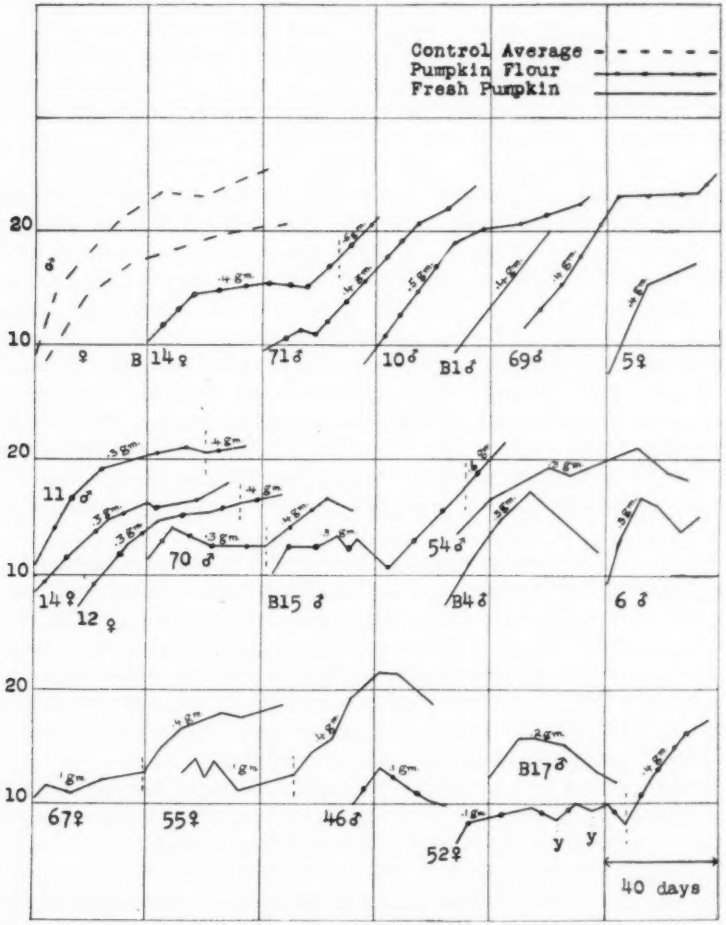

Chart 1. Illustrative growth curves of mice fed pumpkin as sole source of vitamin B.

Age at beginning of experiment:

Control averages: ♂ 28 days; ♀ 26 days.

Pumpkin flour: mouse 52, 26 days; mice 10, B14, B15, 46, 69, 70 and 71, 28 days; mice 11, 12 and 14, 35 days.

Fresh pumpkin: mouse 5, 26 days; mice 6 and B17, 28 days; mice B1 and B4, 34 days; mice 67 and 55, 40 days; mouse 54, 42 days.

of 45 and 92 per cent over the gains of the control averages, were made by two mice which had previously failed to grow on 0.1 gram intake. As a whole this group shows a much better average than the group which consumed 0.3 gram daily, yet 0.4 gram does not stimulate growth satisfactorily for all mice and can not be considered therefore entirely adequate.

The one failure in the group which consumed 0.5 gram daily is again a mouse which maintained this intake from an early age. Throughout the experimental period, mouse 10 gave the appearance of being a mouse in as excellent physical condition as any control and as good or better than any other experimental mouse. Comparison of mouse 10 with Robertson's average would show the mouse to have made superior gains. The gain in weight made by our male controls is so far in excess of the figures given by Robertson, that it seems possible that mouse 10 may have made normal gains in weight. Robertson has shown that gains made by mice are subject to wide variations. The other animals in this group made excellent gains. The amount of 0.5 gram of pumpkin flour eaten daily thus seems to supply mice with enough or nearly enough vitamin B for normal growth.

The two mice, B15 and B14, which consumed an average of 0.6 gram daily made gains far exceeding the gains of the control averages. For adult mice over a short period of time an average daily intake of 0.6 gram of pumpkin flour seems to stimulate growth no better than the 0.5 gram amount. The potency of pumpkin flour would thus be less than one-fifth that of dry brewery yeast.

Vitamin B in fresh pumpkin. The growth curves for mice 67, 55 and B17 show how inadequate 0.1 and 0.2 gram daily intake of fresh pumpkin was in promoting normal growth. On an average daily intake of 0.3 gram, mice 54, B4 and 6 show much better growth than those on a smaller intake, but the growth curves and the figures given in table 1 both show conclusively that 0.3 gram of fresh pumpkin gives an insufficient daily allowance of vitamin B. This failure applies to mice started at this level, as well as to those which had begun at a lower intake.

Both the young mice and those which had failed on an average daily consumption of 0.1 gram, show very satisfactory gains in weight during the period of the experiment when consuming 0.4 gram daily. This amount may thus be considered to yield sufficient vitamin B for normal growth of mice, for a period of five weeks. Fresh pumpkin therefore appears to be one-fourth as potent as dried brewery yeast.

Effect of dehydration on vitamin B. By comparing the gains of the mice fed pumpkin flour with those fed fresh pumpkin (see chart 1 and table 1) it is evident that there is much variability among the pumpkin flour groups and a degree of uniformity between the two fresh pumpkin groups. This may be due to the smaller bulk of the dry food. For a period of about five weeks average daily consumption of 0.5 to 0.6 gram pumpkin flour appears to

stimulate growth in mice as well as 0.4 gram of fresh pumpkin. Smaller amounts of pumpkin flour stimulate growth of mice better than do smaller amounts of fresh pumpkin. The dehydration process here described reduces the vitamin B potency in pumpkin only to a slight extent.

It is interesting to compare this relatively small change in vitamin B content produced by the dehydration of pumpkin with the more serious loss involved in the drying at 70°C. of freshly cooked globe artichoke as previously reported from this laboratory (1). The difference in the drying process used with these two vegetables appears to be chiefly one of ventilation and gradual rise of temperature. The maximum temperature employed in the two is the same and the lengths of the drying periods are not materially different. A considerable difference in the physical properties of the vegetable pulp exists, however, and the preliminary cooking employed in the preparation of our artichoke undoubtedly produced still further changes in the colloidal condition of the plant tissue. Since the acidity and salt content of the two vegetables are widely different as well it is not difficult to imagine more rapid specific catalysis of vitamin B destruction in the artichoke. In any case, this observed difference provides another example of the necessity for individual tests upon vitamin occurrence and lability in apparently similar tissues.

PUMPKIN FLOUR SOURCE OF VITAMIN B FOR RATS. Three young rats which had begun to fail on vitamin B free diet were given 1 gram daily portions of pumpkin flour with immediate and continued normal growth. (See chart 2.) Previously 0.5 gram portions had been tried with only small responses in growth. Similar stimulation of growth to that obtained with 1 gram of pumpkin flour has been demonstrated many times in this laboratory by the administration of 0.3 gram dry brewery yeast. This proportion of practically three to one between the vitamin B potencies of the dry yeast and the dried pumpkin is somewhat less as shown by the use of rats than that shown with mice, four or five to one. While this discrepancy is not large nor in this case particularly important it illustrates the need of uniformity in such vitamin comparisons.

The usefulness of the table first used by the British Medical Research Committee (16) and since modified by numerous others, in which vitamin values are indicated by a series of plus signs, might be greatly increased if figures were substituted wherever common conditions of experimentation obtained, or if the number of plus signs were chosen by some common measure. This would involve the use only of data obtained by the same or comparable means and the adoption of some fairly constant substance as standard. Thus, if the rat feeding method were chosen for the comparison of foods as sources of vitamins A and B, a reasonably generous standard for the former might be taken as 0.1 gram of a potent cod-liver oil and for the latter 0.3 gram of dry brewery yeast.

TABLE 2

Numerical comparison of vitamin B content of certain foods

FOOD	MINIMAL DOSE REQUIRED FOR NORMAL GROWTH				COMPARISON BY SIGN CONVENTION	REFERENCE
	Fresh weight	Dry weight	Percent of standard		Fresh weight	
			Fresh weight	Dry weight	+ = 10^{+}	
	grams	*grams*				
Dry brewery yeast	0.3	0.3	100	100	+++++ +++++	(12)
Globe artichoke, fresh	4.0	0.68	7.5	44	+++++++	(1)
Globe artichoke, canned	4.0	0.6	7.5	50	+++++++	(1)
Globe artichoke, dried	2.0	1.8	15.0	17	$+^{+++++}$	(1)
Dried skim milk	3.0	3.0	10.0	10	+	(17)
Dried pumpkin	1.0	0.92	30.0	32	+++	
Orange juice	12.0		2.5		++	(17)
Fresh milk	11.0	1.3	2.7	22	++	(17)

TABLE 3

Numerical comparison of vitamin A content of certain foods

FOOD	MINIMAL DOSE REQUIRED FOR NORMAL GROWTH				COMPARISON BY SIGN CONVENTION	REFERENCE
	Fresh weight	Dry weight	Per cent of standard		Fresh weight	
			Fresh weight	Dry weight	+ = 10^{+}	
	grams	*grams*				
Codliver oil	0.1	0.1	100	100	+++++ +++++	(12)
Ether extract of orange peel	0.1	0.1	100	100	+++++ +++++	(12)
Cold pressed orange oil	0.2	0.2	50	50	+++++	(12)
Butterfat	0.25	0.25	40	40	++++	(13)
Dried orange peel	0.5	0.46	20	22	++	(12)
Dried lemon peel	1.0	0.8	10	11	+	(12)
Ether extract of lemon peel	1.0	1.0	10	10	+	(14)
Dried skim milk	5.0	4.6	>2	>2	$>^{++}$	(13)
Globe artichoke, fresh	2.0	0.36	5	28	+++++	(1)
Globe artichoke, canned	4.0	0.6	2.5	16	++	(1)
Globe artichoke, dried	0.5	0.47	20	21	++	(1)
Whole blanched walnuts	6.0	5.8	2	2	++	(15)
Fat free walnut meal	2.0	2.0	5	5	+++++	(15)
Alcohol extract of blanched walnuts	2.4	2.4	4	4	++++	(15)
Dried pumpkin	1.0	0.92	10	11	+	

If these standards are each represented by the figure 100 or by a series of plus signs, the value of other foods could be expressed in like terms, provided similar feeding technique were employed in their determination. As an example of the possibilities of this obvious development of the vitamin comparison table, we offer table 2 in which are set forth the vitamin B values of certain foods tested in this laboratory. Only our own results are included since we can vouch for the complete comparability only of experiments made under exactly similar conditions. A similar comparison

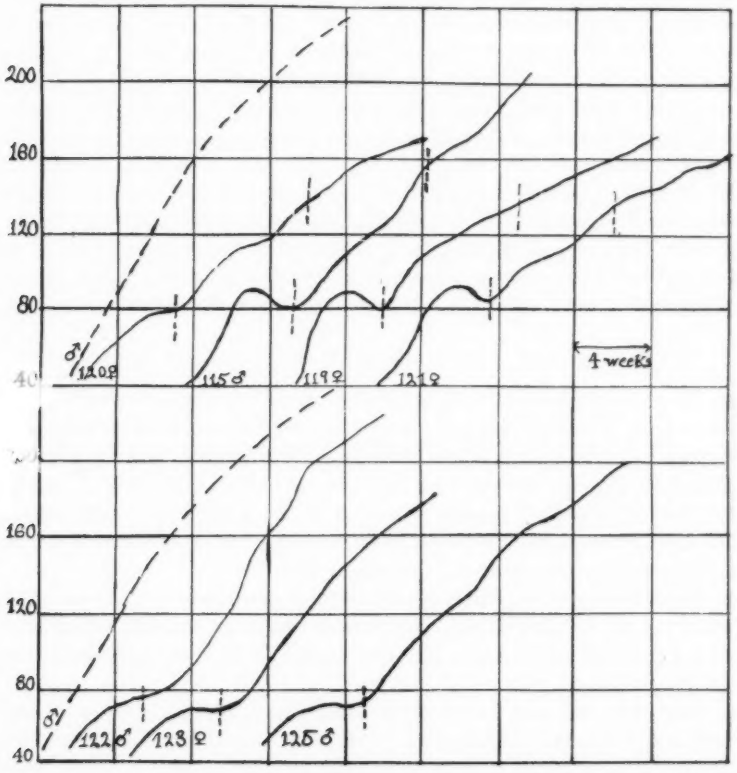

Chart 2. Growth curves of rats fed pumpkin flour as sole source of vitamin A or vitamin B.

Rats 120, 115, 119, 121, were given daily 2 grams of pumpkin flour at first dotted line after decline in weight and xerophthalmia had been established on vitamin A free basal diet. At second dotted line the pumpkin flour was reduced to 1 gram.

Rats 122, 123, 125 were given daily 1 gram of pumpkin flour at dotted line after decline in weight and characteristic symptoms had developed on vitamin B free basal diet.

as to vitamin A content of foods tested under comparable conditions is offered in table 3.

PUMPKIN FLOUR AS SOURCE OF VITAMIN A FOR RATS. Four young rats which were losing weight rapidly and all of which showed severe xerophthalmia because of lack of vitamin A in an otherwise adequate basal diet made excellent return to a normal condition when 1 or 2 grams of dried pumpkin flour was added to their daily ration. The weight curves of these animals are shown in chart 2. Two-gram portions were first given but these were reduced to 1 gram after seven weeks, with no diminution in growth or well-being. It is true that the most rapid period of growth was over at this time and that some storage of vitamin A may have been made upon the previous diet containing 2-gram daily additions of the pumpkin flour. Nevertheless the 1-gram dosage appeared to be adequate for maintenance although perhaps not for recovery from a severe drainage of vitamin A in young animals.

The relative richness of pumpkin solids in vitamin A is not surprising in view of the numerous reports of similar potency in other yellow pigmented vegetable foods. Indeed, a definite relationship between vitamin A and the character of lipochrome present in all the foods studied in this laboratory appears to exist. An investigation upon this point is now under way.

SUMMARY

1. As the sole source of vitamin B, pumpkin flour prepared by the method here described, has been found inadequate as a growth promoting factor for mice in amounts less than three-tenths gram daily. Partially satisfactory growth resulted from an average daily intake of 0.3 and 0.4 gram, and satisfactory growth from 0.5 to 0.6 gram. For rats, 1-gram daily doses were found sufficient to restore a practically normal growth rate after decline on vitamin B-free basal diet.

2. Fresh pumpkin was found satisfactory as source of vitamin B in stimulating the growth of mice when consumed in an amount which averaged 0.4 gram of solids daily, but was unsatisfactory when consumed on the 0.3 gram level.

3. Tested by the gain in weight of albino mice, pumpkin has a vitamin B potency of one-fifth to one-sixth in the dehydrated form and one-fourth in the fresh form of the growth promoting qualities of dry brewery yeast. Tested by gain in weight of young rats the potency of the dehydrated pumpkin is apparently one-third to one-fourth that of dry brewery yeast.

4. Pumpkin, dehydrated at a low temperature for a long period of time, with a large volume of air constantly passing through the dryer, appears to suffer only a slight loss of vitamin B. Attention is called to the difference between this result, and that previously reported from a study of the destruction of vitamin B in dried globe artichoke.

5. The dehydrated pumpkin prepared by the process described restores normal conditions in young rats suffering from vitamin A deficiency when fed at a 2-gram level, and maintains such conditions in growing rats when fed at a 1-gram level. The coincidence of this vitamin A potency with a high lipochrome content in the pumpkin furnishes another significant instance of some correlation between these vegetable properties.

6. A numerical comparison of the vitamin B and vitamin A values of a number of foods investigated in this laboratory is offered as a suggestion of the possibilities resulting from the adoption of a uniform feeding procedure.

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FURTHER STUDIES ON COTTON SEED MEAL INJURY[1]

ICIE G. MACY AND JULIA P. OUTHOUSE

From the Department of Household Science of the University of California, Berkeley

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The use of cotton seed meal in feeding domestic animals dates back to the middle of the nineteenth century, when the oil was first successfully expressed from the kernels thus leaving the press-cake as a by-product. Although the knowledge of a high mortality among certain animals, when fed the cotton seed rations, has prevailed since the residue was made available in commercial quantities, the cause has only been known for a short time. The young and growing animals of most species are particularly susceptible to the so-called cotton seed injury, even though the meal is fed in limited quantities for a short period of time.

In view of its composition and irrespective of its deleterious properties, cotton seed meal has been bolted and milled into a palatable brand of cotton seed flour,[2] (3) light yellow in color, fine in texture, with a pleasant nutty flavor. It is alleged by some people in the cotton belt that this flour has been used as an article of diet by some families for a number of years. However it has not been used generally by the public owing to the fact that its fitness for food has been questioned on account of the ill effects resulting from the feeding of the meal to live stock. The literature is devoid of records on human mortality from cotton seed poisoning though one hears rumors of blindness and insanity among individuals who, without any previous knowledge of its ill effects upon animals, are accustomed to using large quantities of cotton seed flour as a standard

[1] A preliminary report of this paper was presented at the meeting of the Society of Pharmacology and Experimental Therapeutics held in St. Louis, Mo., December 27 to 29, 1923. Part of the expenses of this investigation was defrayed by a grant from the Research Board of the University of California.

[2] Composition of cotton seed flour given by G. S. Fraps, Chemist, Texas Agricultural College:

	per cent
Protein	51.19
Fat	11.40
Crude fiber	3.05
Nitrogen-free extract	22.22
Water	6.14
Ash	6.00

article of diet. In view of the fact that there is a toxic substance (1a) present in cotton seed meal which is not removed by the milling processes, it would seem unwise to use even small quantities of cotton seed flour, until commercial methods have been devised to remove the poisonous principle or at least to insure its complete destruction.

The cause of cotton seed injury is now attributed to the presence of gossypol, the yellow pigment, which has phenolic properties. This compound has been isolated and studied by Withers and Carruth (11), (11a), (11b) and Carruth (2). These investigators and more recently Alsberg and Schwartze (1), (1a), (1b) have demonstrated by conclusive feeding experiments that gossypol produces upon animals poisoning effects similar to toxic cotton seed meal. Despite the widespread use of cotton seed meal for feeding domestic animals and the numerous investigations upon the cause and effect of its deleterious properties upon certain individuals, data recording the physiological changes that accompany cotton seed injury are limited.

Jones and Waterman (4) have recently demonstrated that

> the addition to the protein of 1 per cent of its weight of the toxic principle, gossypol, known to be present in cotton seed kernels to the extent of from 1.5 to somewhat more than 5 per cent of the estimated (N × 6.25) protein content, interferes markedly with the digestion *in vitro* of the cotton seed globulin by pepsin and trypsin. The incomplete digestion (83 per cent by animals) of the protein content of cotton seed press-cake preparations is tentatively explained by an inhibitive effect of gossypol.

The results of these experiments together with the fact that several investigators have reported a poor utilization of the protein in cotton seed meal, give direct evidence that gossypol is at least one of the limiting factors and interferes with digestion in the animal body. In the present investigation an attempt has been made to obtain a clearer understanding of the responses of the body to cotton seed meal, as well as to gossypol feeding. This information would be desirable as an aid in elucidating the pathological processes that are brought about during the consumption of such products.

PLAN AND METHODS OF THE PRESENT INVESTIGATION. It was reported in previous studies (5), (6) that rabbits lived 5 to 35 days; guinea pigs lived 5 to 41 days; pigeons lived 7 to 80 days; and mice lived 4 to 180 days when fed upon cotton seed meal diets which were made adequate in respect to all the essential components by supplemental additions. Although these animals developed cotton seed poisoning readily, they were not considered suitable subjects for metabolic studies over a long period of time.

The dog was selected for these physiological studies first, because it was thought that cotton seed injury would develop more slowly thus giving a longer transition period for observation, secondly, this animal is

easily handled. Blood can be drawn with great ease and with little discomfort and disturbance to the animal; the excreta can also be collected with appreciable accuracy.[3] Moreover dogs are hardy animals, and do not succumb readily to kidney disturbances and to infectious diseases, therefore minimizing the chance of error in the histological and pathological observations. Young animals were selected because the growing organism is more susceptible to the so-called cotton seed injury. Three female dogs were used in this series of studies. Two were fed upon a cotton seed meal diet over a period of twenty-six weeks during which time frequent blood and urine analyses were made. The third dog was fed daily until death on an adequate ration plus 25 mgm. of gossypol acetate per kilogram body weight; frequent blood determinations were made.

Feeding of cotton seed meal.[4] Dog I, a playful short hair mongrel, approximately four months old, was in seemingly excellent physical condition, possessing a sleek coat, surplus fat and a ravenous appetite. Dog II was a young but full-grown animal with a long hair coat, in good health, yet possessing a delicate appetite and a sullen disposition. Both dogs were fed alike on the cotton seed meal ration[5] plus 100 cc. of tomato juice[6] daily to insure a liberal supply of accessory substances in the diet. Fresh food was mixed, weighed, and fed in the morning and the animals were permitted to eat *ad libitum*. The following morning the food pans were weighed and the food intake calculated by difference. Body weights were recorded practically every third day. Water was kept in the cages

[3] The authors take this privilege of expressing their appreciation to Prof. Guy Clark, Department of Biochemistry and Pharmacology, the University of California and to Prof. Lafayette B. Mendel of Yale University for many valuable suggestions offered during the progress of this work.

[4] Cotton seed meal IV was kindly furnished by R. K. Wootten of the Chickasha Cotton Oil Company, Chickasha, Oklahoma.

[5] Composition of cotton seed meal basal diet:

	grams
Cotton seed meal IV	45
Starch	20
Crisco	25
Salt mixture[6]	4
Butter	6

[6] Karr, W. G., Journ. Biol. Chem., 1920, xliv, 256.

Salt mixture:

Sodium chloride	10
Calcium lactate	4
Magnesium citrate	4
Ferric citrate	1
Few drops of I K I	

all the time, and at no time could the hygienic condition be questioned, for the room was kept clean, well ventilated and lighted, by a south and west exposure. During the periods in which the nitrogen balances were being determined the dogs were kept in metabolism cages, otherwise they were kept in the same room in pens 3×5 feet where it was possible for them to exercise freely. Each morning they were permitted to exercise for an hour or so in the sunlight in a large out-door pen.

The dogs ate freely of the cotton seed meal diet (charts 1 and 2) and gained in weight for a period of approximately 145 days, again demonstrating that the protein of cotton seed meal is adequate in respect to the amino acids which are essential for maintenance. These results corroborate the studies of Osborne and Mendel (9), Richardson and Green (10), Nevens (8) and others on rats and mice. At the end of 192 days it was necessary to bring the experiment to an end although both animals appeared in good health with the exception of extreme soreness in the abdominal regions. It was impossible to pat the dogs in this portion of the body, much less lift them up without causing them great distress as exhibited by barking and growling.

Throughout the entire investigation, frequent blood analyses were made by the Folin and Wu methods (12) and the recent modifications (13) evolved from them. These data are reported in tables 1 and 2. The first blood analysis of these series recorded on April 12 was made while the animals were on a control basal diet. The results obtained during the following experimental period indicates no change in the chemical constituents of the blood due to the cotton seed ration. This is not in accord with the work of Menaul (7) on sheep. He has reported an increase above normal followed by a decrease below normal in both the non-protein nitrogen and sugar constituents of the blood. The relatively high proportion of the blood constituents reported for dogs I and II on July 19 is considered to be due to environmental changes—excessively warm weather during the week. Chemical analysis of the food intake and the excreta at various periods during the development of cotton seed meal injury show that there was a positive nitrogen balance in the early weeks of the experiment whereas after the onset of the malady there was a decided negative balance (tables 3 and 4). This would corroborate the results of Alsberg and Schwartze (1) in that they found an impaired digestion (decrease in absorption) of nitrogen, and would substantiate the investigation of Jones and Waterman (4) who found that gossypol inhibits peptic and tryptic digestion. A slight continuous decline in body weight was observed simultaneously with the decreased nitrogen absorption.

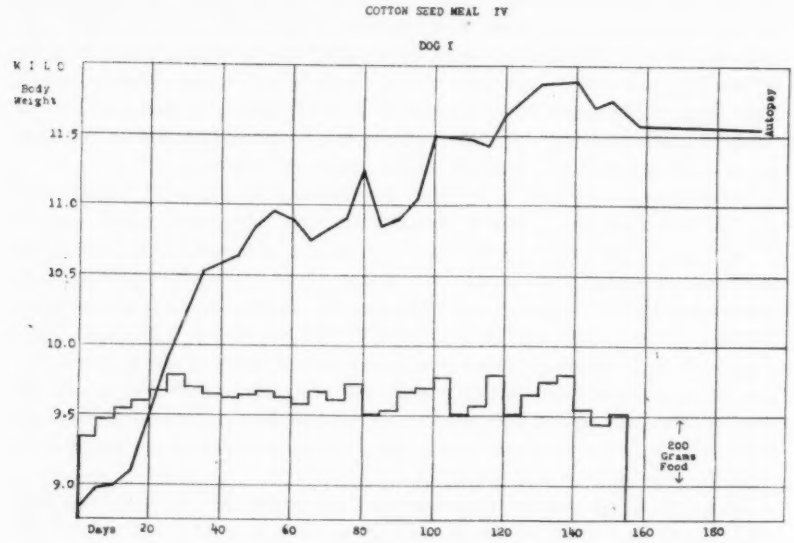

Chart 1

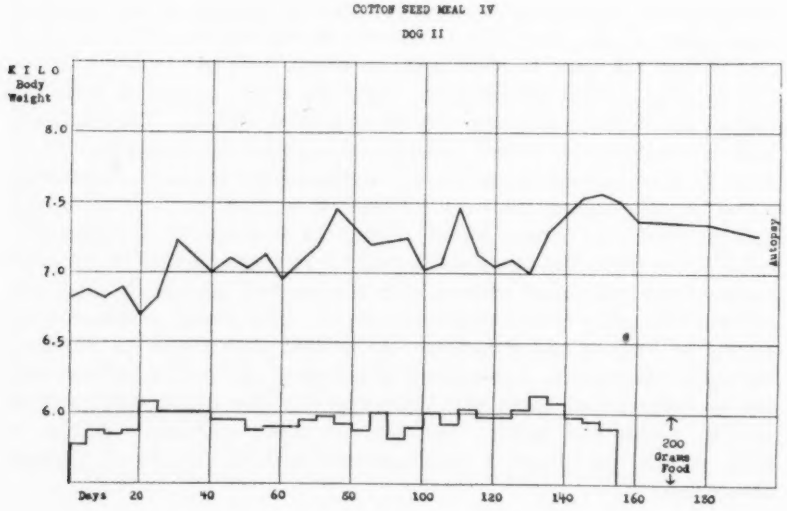

Chart 2

Dogs I and II were etherized and autopsied[7] immediately on the 192nd day of the experiment. The results (protocol) showed macroscopic similarity. In both cases the dogs were well nourished, dog II exhibited a great excess of soft fat; the intestinal canals were hyperemic,

TABLE 1

*Blood analysis**

Dog I

DATE	NON-PROTEIN NITROGEN	UREA	URIC ACID	SUGAR	CREATIN PLUS CREATININ	CREATININ	CREATIN
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
April 12	24.5	8.1	1.81	85.0	4.13	1.31	2.82
April 19	23.5	7.17	2.22	103.0	3.69	1.33	2.36
May 9	26.9	9.8	1.69	108.0	5.10	1.33	3.77
May 18	23.0	8.1	1.56	124.0	4.42	1.40	3.02
May 31	21.8	8.2	1.96	117.0		1.32	
June 14	21.9	7.8	1.60	122.0	4.60	1.40	3.20
July 2	26.9	10.4	1.80	113.0	6.00	1.30	4.70
July 19	57.0	32.6	2.65	138.8		1.31	
August 2	27.0	9.9	2.05	113.0	3.4	1.46	2.94
September 22	26.9	8.2	3.36	155.0	6.55	1.63	4.92

* Reported in milligrams per 100 cc. of blood.

TABLE 2

*Blood analysis**

Dog II

DATE	NON-PROTEIN NITROGEN	UREA	URIC ACID	SUGAR	CREATIN PLUS CREATININ	CREATININ	CREATIN
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
April 12	26.5	9.9	1.74	84.7	4.56	1.24	3.32
April 19		10.7		87.3			
May 9	40.3	16.2	2.03	131.0	5.53	1.63	3.90
May 18	28.8	10.4	1.86	111.0	4.50	1.55	2.95
May 31	24.0	8.6	1.50	101.0		1.00	
June 14	22.7	10.0	1.40	101.0	4.90	1.40	3.50
July 2	29.2	11.7	2.1	109.0	4.50	1.50	3.00
July 19	40.4	cloudy	2.72	133.3		1.42	
August 2	30.1	11.3	2.06	96.1	3.69	1.60	2.09
September 22	35.0	11.3	3.48	130.0	4.30	1.89	2.41

* Reported in milligrams per 100 cc. of blood.

[7] The authors gratefully acknowledge the assistance of Prof. T. D. Beckwith of the Department of Bacteriology and Experimental Pathology of the University of California in making careful post-mortem examinations of the animals used in this investigation.

TABLE 3

DATE	WEIGHT OF DOG I	FOOD CONSUMED* PER KILO BODY WEIGHT		URINE ANALYSIS PER KILO BODY WEIGHT							FECES KILO BODY WEIGHT	
		Basal diet†‡	N intake	Volume	Specific gravity	Urea-N	NH_3-N	Creatin-N	Creatinin-N	Total-N	Feces	total-N
Period I												
	kilos	*gram*	*gram*	*cc.*		*gram*	*gram*	*gram*	*gram*	*gram*	*gram*	*gram*
July 27	11.53	20.1	0.609	58.1	1.019	0.155	0.030	0.018	0.033	0.360	12.20	0.116
July 28		18.0	0.542	49.4	1.022	0.179	0.027	0.054	0.034	0.326	26.15	0.090
July 29		19.1	0.577	45.7	1.025	0.116	0.022	0.040	0.034	0.348	8.50	0.072
July 30	11.59	22.4	0.676	54.4	1.024	0.097	0.023		0.046	0.382	28.99	0.194
Average	11.56	19.9	0.601	51.9	1.023	0.137	0.026	0.033	0.037	0.354	18.96	0.188
						Nitrogen balance +0.129						
Period II												
September 7	11.76	12.59	0.384	24.66	1.028	0.076	0.011	0.152	0.271	0.324	6.46	0.050
September 8		17.35	0.528	37.42	1.029	0.264§		0.176	0.286	0.388	9.78	0.079
September 9	11.88	19.61	0.595	54.71	1.020	0.228	0.006	0.123	0.305	0.491	33.67	0.261
September 10		20.71	0.628	58.92	1.020	0.323	0.006	0.230	0.379	0.520	8.59	0.552
Average	11.82	17.57	0.527	43.93	1.024	0.223	0.008	0.170	0.310	0.431	14.85	0.236
						Nitrogen balance −0.140						

* 100 cc. tomato juice = 0.037 gram N fed daily.

† Basal food mixture:

	grams
Cotton seed meal (42 per cent protein)	45
Starch	20
Crisco	25
Butter	6
Salt mixture‡	5

‡ Karr: Journ. Biol. Chem., 1920, xliv, 256.

Salt mixture:

	grams
Sodium chloride	10
Calcium Lactate	4
Magnesium citrate	4
Ferric citrate	1

Few drops of I KI

§ Urea + NH_3.

TABLE 4

DATE	WEIGHT OF DOG II	FOOD CONSUMED* PER KILO BODY WEIGHT		URINE ANALYSIS PER KILO BODY WEIGHT							FECAL ANALYSIS PER KILO BODY WEIGHT	
		Basal diet†‡	N intake	Volume	Specific gravity	Urea-N	NH_3-N	Creatin-N	Creatinin-N	Total-N	Feces	total-N
					Period I							
	kilos	*gram*	*gram*	*cc.*	*gram*	*gram*	*gram*	*gram*	*gram*	*gram*	*gram*	*gram*
July 27	7.45	8.10	0.249	44.40	1.015	0.109	0.015	0.035	0.026	0.217	1.006	0.001
July 28		9.13	0.281	22.95	1.030	0.099	0.006	0.023	0.023	0.135	4.362	0.018
July 29	7.47	14.32	0.448	17.14	1.018	0.036	0.006	0.010	0.017	0.116	2.390	0.019
July 30		0.0	0.005	32.12	1.018	0.078	0.013		0.026	0.196	2.971	0.027
Average.....	7.46	7.64	0.246	29.15	1.025	0.081	0.010	0.023	0.023	0.166	2.682	0.016
						Nitrogen balance +0.064						
					Period II							
September 7	7.61	10.91	0.335	38.76	1.020	0.209	0.040	0.029	0.027	0.441	0.605	0.056
September 8		5.65	0.176	15.51	1.028	0.095	0.039	0.017	0.015	0.211	1.104	0.080
September 9	7.52	18.09	0.553	57.18	1.021	0.247	0.075	0.019	0.017	0.465	3.258	0.025
September 10		10.12	0.311	34.84	1.021	0.200	0.025	0.013	0.023	0.328	1.822	0.139
Average.....	7.57	11.19	0.344	36.57	1.023	0.188	0.045	0.020	0.021	0.381	1.697	0.075
						Nitrogen balance −0.112						

* 100 cc. tomato juice = 0.037 gram N fed daily.

† Basal food mixture.

	grams
Cotton seed meal (42 per cent protein)	45
Starch	20
Crisco	25
Butter	6
Salt mixture‡	4

‡ Karr: Jour. Biol. Chem., 1920, xliv, 256.

Salt mixture:

	grams
Sodium chloride	10
Calcium lactate	4
Magnesium citrate	4
Ferric citrate	1

Few drops of I + K I

the mesenteric vessels engorged, intestines hemorrhagic with excess amount of mucus and possibly a sloughing off of the mucosa, the heart dilated and the kidneys congested. All of these findings are characteristic of cotton seed meal injury.

No attempt was made to analyze cotton seed meal IV chemically for its gossypol content. However, it was moistened with molasses and fed to two mature rabbits, for they are animals which are particularly susceptible to cotton seed poisoning. Toxic symptoms developed within twelve days, thus demonstrating a comparatively potent product.

Jones and Waterman (4) state that

> the gossypol content of unheated cotton seed press-cake preparations can not be definitely stated. Experiments with whole ground cotton seed, however, indicate a yield of gossypol actually isolable from that product amounting to from 1.5 to 5.25 per cent of the protein content.

Dog I had consumed approximately 21 kgm. of cotton seed meal IV up to the termination of the experiment; dog II had consumed only about 3.5 kgm. over the same period. Macroscopic post-mortem findings (protocol) demonstrate a more severe condition in the case where a larger quantity of cotton seed meal was eaten. These results will be checked by microscopic studies and reported later.

Feeding of gossypol acetate.[8] Dog III, eighteen weeks old, was a vivacious and healthy bull terrier possessing a ferocious appetite. She was given a diet of dog biscuits together with fresh milk or milk powder, tomato juice and frequently a few grams of ground meat. Gossypol acetate was weighed out and fed separately in gelatin capsules to insure a constant daily intake of 25 mgm. per kilo body weight. Food was not given for an hour or so after the gossypol had been taken, in order that plenty of time should be given for it to pass from the stomach into the intestine. Gossypol tends to nauseate the individual, and the food as well as the deleterious substance itself may be ejected. Some food was eaten at each feeding but the appetite varied, for on some days liberal quantities of food were consumed, while on other days only a small portion. It was impossible, however, to weigh the food intake accurately because of excessive diarrhea and sometimes ejection of food. Although the appetite of the dog fluctuated throughout the experiment, nevertheless there was no time at which all food materials were completely refused, thus eliminating the inanition factor in the final results. Furthermore there was no appreciable loss in body weight (chart 3).

[8] Dr. E. W. Schwartze, Pharmacologist in charge, Pharmacological Laboratory, Bureau of Chemistry, Washington, D. C., kindly furnished the gossypol acetate used in this investigation.

Frequent blood analyses were made with no significant results (table 5). It was impossible to analyze the excreta because of the diarrhea that was prompted by the gossypol. Dog III died on the twenty-sixth day of the experiment, consequently giving too short a period for extensive metabolic observations; yet the pathological studies are of much value in confirming

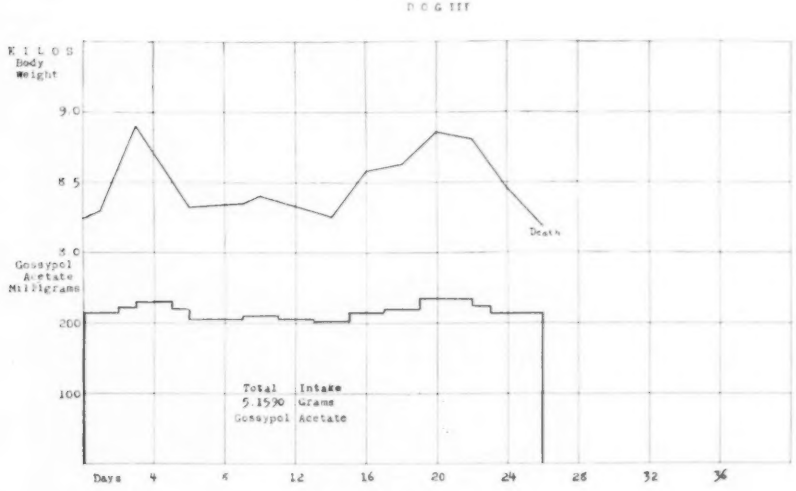

Chart 3

TABLE 5

*Blood analysis**

Dog III

DATE	NON-PROTEIN NITROGEN	UREA	URIC ACID	SUGAR	CREATIN PLUS CREATININ	CREATININ	CREATIN
1923	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
May 10†	52.4	10.6	2.14	124.0	5.5	1.1	4.4
May 31	25.8	10.0	1.74	119.0		1.3	
June 5	25.6	11.8	1.75	136.0		1.4	
June 14	25.5	11.0	1.40	132.0	4.7	1.17	3.53

* Reported in milligrams per 100 cc. of blood.

† Analysis made the day before experiment was started.

the similarity of results from the feeding of cotton seed meal with those from feeding gossypol.

The animal was active until death, though a disturbance of health was evidenced by the hair coat and eyes a few days before death. The hair lacked luster and was unkempt; the eyes appeared weak and mucous

material exuded from them. Nevertheless the dog was always ready to jump out of her cage and to participate in strenuous play in the outdoor pen. On the morning of the twenty-sixth day of the experiment the daily dose of gossypol was given, together with a few pieces of dog biscuit, and even though the dog ate voraciously, it appeared difficult for her to chew hard food material. An hour later she dropped dead. The autopsy,

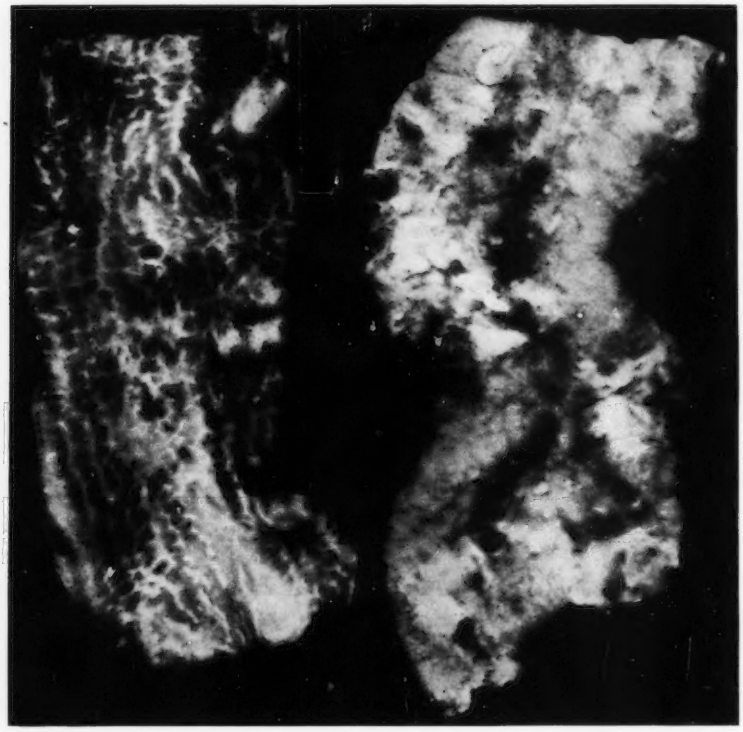

Fig. 1. Macroscopic appearance of intestine of the dog which died from the effects of gossypol acetate. Note the deep hemorrhagic areas and the sloughing off of the mucosa.

which was made shortly after death ensued, revealed the typical macroscopic post-mortem findings of cotton seed meal injury (protocol). However, this was an acute case of poisoning and more severe as was shown by the large amount of light yellow fluid in the abdominal cavity, the extreme congestion of all splanchnic organs, as well as the extensive deep fresh hemorrhagic areas in the intestinal canal (fig. 1).

Dog III consumed 5.159 grams of gossypol acetate or 4.638 grams of free gossypol (Carruth (2) found that gossypol acetate contained 10.1 per cent of acetic acid) over a period of twenty-six days. Withers and Carruth (11a) have found that there is no difference in the action of the "acetate" and the "free" gossypol. The necropsies observed after feeding gossypol are similar to those obtained by feeding toxic cotton seed meal.

SUMMARY

1. Cotton seed meal diets when fed for a long period of time, have untoward effects upon the dog. These observations are in accord with our previous studies on the physiological value and toxicity of cotton seed products.

2. The protein of cotton seed meal has proven adequate for maintenance and also growth of the dog.

3. Dogs eat cotton seed meal diets freely and gain in body weight. Chemical analyses of the food intake and the excreta at various periods during the development of cotton seed meal injury show that there may be a positive nitrogen balance in the early weeks of the experiment whereas after the onset of the disease there is a distinct negative balance. This perhaps indicates that gossypol impairs the digestion of food materials *in vivo*.

4. Daily doses of 25 mgm. of gossypol acetate per kilogram body weight when fed in an adquate diet proved fatal to the dog. Macroscopic postmortem observations revealed apparent lesions similar to those generally found in cotton seed meal injury; namely, excess abdominal fluid, congestion of all the splanchnic organs, and hemorrhagic intestines.

5. Frequent blood analyses were made during the progress of cotton seed meal injury but these results were not significant.

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PROTOCOLS

Macroscopic observations

	AUTOPSY		
	Dog I	Dog II	Dog III
Body weight	11.55 kilos	7.38 kilos	8.24 kilos
Nutrition	Excellent—excess fat	Large excess soft fat	Good but no excess of visible fat
Hair coat	Sleek	Sleek	Unkempt
Abdominal cavity	Hyperemia in intestinal canal; no visible exudate; mesenteric vessels engorged	Dilatation of circulatory vessels in mesentery; no exudate	Large excess of yellow serous fluid; no distension; general congestion and engorgement of mesenteric vessels
Small intestine	Hyperemic; very hemorrhagic throughout; lumen hemorrhagic excessive amount of mucus with possible sloughing off of mucosa	Hyperemic; hemorrhagic and congested areas; contained yellow fluid	Congested and very hemorrhagic; deep seated hemorrhages both new and old; mucosa greatly thickened with sloughing off, excess bloody mucus; contained yellow liquid material
Colon	Congested and slightly hemorrhagic	Upper portion hyperemic	Very hemorrhagic to anus; filled with yellow liquid fecal matter
Stomach	Empty; some mucus	Empty; apparently normal	Partially filled with food material; particles of gossypol present; congestion in general; reddened areas but capillaries do not appear broken; mucus present but not in great excess
Heart	Right side dilated otherwise normal	Appears normal	Right side greatly dilated and engorged; left auricle contained a small vegetative growth
Kidneys	Capsule normal; cortical layers congested; left kidney weighed 36 grams	Small; capsules not adherent; in medulla there are reddened areas and engorgement in this portion of the left kidney; cortex apparently normal; weight of each kidney—15.3 grams	Right kidney weighed 36.7 grams; capsule not adherent; congested cortex. Left kidney weighed 35 grams; capsule slipped off quite easily with the exception of a few small spots which adhered; upon cross section marked congestion was apparent

Adrenals	Apparently normal	Normal	Normal
Liver	Apparently normal	No enlargement; lobes prominent	Large and greatly congested
Gall bladder	Congested wall; distended	Engorgement of walls	Partially filled with bile; deposited on wall were yellow particles; general congestion
Spleen	Slight congestion	Normal	Normal
Pancreas	Apparently normal	Normal	Normal
Lungs	Very slight hemorrhagic areas	Greatly congested	Slight congestion; tip of lobe of left lung very slightly hemorrhagic but contained air and no solid areas were found
Bladder	Normal	Enlarged blood vessels	Normal
Teeth and gums	Normal	Normal	Gums pale; teeth firm
Bones	Normal	Normal	No enlargements nor fragility

ON WEICHARDT'S SUPPOSED "FATIGUE TOXIN"

FREDERIC S. LEE AND B. ARONOVITCH

From the Department of Physiology, Columbia University, New York

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Weichardt (1), in 1904, put forth the most remarkable ideas concerning the causation of fatigue that had been suggested since the publication, in 1865, of Ranke's identification of certain "fatigue substances." On the basis of a large amount of experimental work Weichardt claimed that there is formed in fatigue not simply the usually accepted fatigue substances, but a more specific substance, a true "fatigue toxin," analogous to bacterial toxins, and that this is chiefly responsible for fatigue.

A little later than his first experiments he claimed that a substance, or substances, presumably identical with fatigue toxin and possessing similar fatiguing properties, can be obtained from proteins outside the living body, such as egg albumin and the proteins of the press-juice of non-fatigued muscles, by such diverse chemical and physical means as *1*, reduction methods, by sodium sulphite, sodium nitrite and nascent hydrogen, obtained through the agency of colloidal platinum, the negative electrode, and other methods; *2*, oxidation methods, by dilute nitric acid, hydrogen peroxide, the positive electrode, etc.; and *3*, hydrolysis. The product so obtained he named "kenotoxin." He also mentions as sources of kenotoxin urine, the excrement of birds, expired air, plant proteins and bacterial endotoxins.

However obtained, Weichardt claimed that his toxin is capable of forming within the tissues of animals its own antidote, a "fatigue antitoxin," which will prevent the appearance, or, if already present, will eliminate the customary phenomena, of fatigue. Other chemical substances, such as succinimide and certain guanidine derivatives, possess similar antitoxic properties. His belief in the value of his antitoxin, or antikenotoxin, was indicated by the fact that he even went so far as to apply for patents on this product, in Germany in 1903 and in the United States in 1904. The latter was granted two years later, and the official record of the transaction may be found in the report of the U. S. Patent Office (2).

Weichardt has had a few followers, some of whom have presented apparently confirmatory experimental evidence of his conclusions, but some of whose results are fantastic. He has also had certain worthy opponents (3).

In his various publications on fatigue the present senior author's attitude has been one of scepticism of Weichardt's ideas and of a growing belief that fatigue is not a specific phenomenon, caused by a few specific metabolic products, still less by a specific toxin, but that it is a convenient name for the depression of the working power, following any one of many metabolic, or even certain physical, changes in the organism, and that many metabolic substances deserve the name "fatigue substances." We have, therefore, made an experimental test of Weichardt's hypothesis, in so far as it pertains to the production of a fatigue toxin within the animal organism by the performance of muscular work. The reader of Weichardt's successive papers finds occasional contradictions in his statements of experimental results and an ever-increasing complexity of experimental procedure. We have endeavored to simplify the problem and the method and to base our work on the propositions that, if a fatigue toxin exists, it exists, and ought to be capable of demonstration, in the muscles that produce it, and it does not exist in non-fatigued muscles. We published a brief preliminary report of our conclusions in 1917 (4); the presentation of our detailed results has been delayed till the present time. Although our experiments are not very numerous, we believe that the uniformity of the results justifies their publication. Because of our inability to obtain evidence of a natural production of "fatigue toxin" we have not deemed it necessary to proceed to its artificial production or to the production of its antibody.

The method. Our general method has been to fatigue animals, to press out the juice from the skeletal muscles, to filter this juice and inject it into other animals or administer it to excised muscles, and to observe the effects.

As the source of the muscle juice we have used cats chiefly; rabbits were employed in some of the earlier and less satisfactory experiments. The animals were fatigued by being allowed to run in a vertical wheel, 0.914 m. in diameter, constructed of a wooden frame covered by wire netting, the inner circumference forming a running track 2.87 m. long and 20 cm. wide, which was revolved about a horizontal axis by an electric motor. The rate of revolution was adjusted to the disposition of the individual animal to run and varied from 3 times per minute with rabbits to 8 to 15 times with cats. The duration of this fatiguing process varied greatly and its termination was dependent upon a disinclination of the animal to run longer. It was then removed from the wheel and immediately killed by decapitation. By suspension of the body as much blood as possible was made to flow from the severed vessels. In most cases a wire electrode was then placed within the skin around the posterior end of the trunk of the body and another around the ankles, and a tetanic stimulating current was sent through the muscles of the hind legs for

the purpose of causing further fatigue. This was continued intermittently, —usually for from three to ten minutes—until the muscles ceased to contract to the strongest currents that are customarily used for muscular stimulation. The muscles were then removed, cut up by scissors, placed in a mortar, and thoroughly ground with kieselguhr into a pulpy mass. This was folded within several layers of cheese-cloth, placed in a strong steel vise, and subjected to a very heavy pressure. In this manner a quantity of muscle juice was obtained, amounting often to more than 10 cc. This was filtered through filter paper and the juice so obtained was usually employed at once for injection into animals or for administration to muscles as described below. It was always found to be strongly acid in reaction to litmus. The successive processes in obtaining the juice were usually performed aseptically. The time elapsing between death and the completion of the preparation of the juice for injection varied in successive experiments from sixty-three minutes to five hours and eight minutes (in one extreme case), but averaged approximately two and a half hours.

There is no doubt that our animals were thoroughly fatigued in the physiological sense. When taken from the wheel they were breathing very rapidly. Within a short time, often within five to eight minutes, after decapitation the muscles went into pronounced rigor.

The action of muscle juice on animals. We have observed the general action of the juice of fatigued muscles on normal animals. Guinea pigs were usually employed as the test animals, although cats and rabbits have served occasionally. The injections were made sometimes intravenously, sometimes subcutaneously, but in the majority of the experiments intraperitoneally. Usually about 5 cc. of the juice were injected at first, and an additional 5 cc. a few minutes later.

Weichardt observed that when he injected into animals the juice of fatigued muscles, or the artificially prepared kenotoxin, he obtained three distinct effects: stupor, a lowering of the body temperature, and a slowing of the respiration which often ended in death. We have therefore paid especial attention to these phenomena.

In our experiments stupor customarily followed the injection of the juice. The animal became quiet and continued so for hours, often apparently dozing off to sleep.

The most striking effect, however, was a fall of the body temperature. Rectal temperature was observed just before the juice was injected, again usually fifteen minutes after the completion of the injection, and at fifteen-minute intervals thereafter. The fall began promptly after the injection, and the lowest reading of the thermometer was obtained during the first half-hour. Of the ten experiments in which the body temperature was observed, the average maximum fall in the five in which

the observations were most frequent was 1.1°C., the greatest being 1.6°. Immediately after the lowest point was reached the temperature began to rise, and there was a slow gradual return toward the normal. Weichardt reported a continuous fall in temperature and one of several degrees, until death usually intervened. In all of our cases there was the fall followed by the rise. Table 1 presents the data from our five most complete experiments. In all of these, cats served as the source of the muscle juice and guinea pigs as the test animals.

Counts were made of the rate of respiration immediately before and at intervals after injection. The results were not constant, there appearing sometimes an increase and sometimes a decrease, and no inference, beyond the fickleness of a rabbit's or a guinea pig's breathing, can be drawn from the figures.

Of nineteen animals injected, five died. Of these three struggled strongly upon the administration of the juice, gave a few gasps and ex-

TABLE 1

Action of juice of fatigued muscles on body temperature

NUMBER OF EXPERIMENT	INITIAL TEMPERATURE	FALL OF TEMPERATURE AFTER INJECTION, 15 MINUTE INTERVALS								MAXIMUM FALL
		15	30	45	1 hour	15	30	45	2 hours	
	°C.									
15	37.7		0.9		0.7		0.7		0.1	0.9
16	38.6	0.2	0.6	0.5	0.4	0.2	0.1	0	0.6	0.6
17	38.9	1.1	0.8	0.7	0.8	0.4	+0.1	0.1		1.1
18	38.6	1.6	1.4	1.3	1.2	1.1	1.2	1.1	1.1	1.6
22	39.0	0.8	1.2	1.0	1.0	0.8	0.6	0.4	0	1.2

pired at once. In all of these cases the juice was introduced directly into the jugular vein, and the possibility of an embolus was not excluded. In the two other animals, which were injected intraperitoneally, death occurred on the following day, and the autopsy revealed a caked granular mass, apparently the coagulated muscle proteins, surrounding and among the abdominal viscera at the site of the injection.

It is evident that none of the results so far presented are really contradictory of Weichardt's findings or are necessarily opposed to an interpretation of the presence of a fatigue toxin. It should be observed, however, that none of the positive results is specific: Stupor is one of the most common effects of injecting foreign, non-irritating substance into animals; a fall of temperature, especially after the injection of proteins, is well recognized (5); and an occasional death from the introduction of foreign substances into the body through either the intravenous or the intraperitoneal route is to be expected.

The desirability of performing a series of control experiments, in which non-fatigued muscles were used as the source of the juice, seems too obvious

to mention, but a careful search of Weichardt's papers fails to reveal that he performed adequate controls. Rare mention is, however, made of the fact that the juice of fresh muscles proved to be non-toxic or non-fatiguing. We have endeavored to fill this gap, and the significant feature of our work here is that *results similar to those reported above were obtained when the muscle juice from non-fatigued control animals was injected.* This juice was prepared in the same manner as the fatigue juice, except that the animals were not previously fatigued, either by running in the wheel or by the electrical stimulation of their muscles. It was observed that the pulpy mass of muscle, after grinding, was paler and more viscous and yielded juice with greater difficulty than the mass of fatigued muscle. The non-fatigue juice, like that from the fatigued muscles, was acid to litmus. When injected into guinea pigs, there followed stupor, a fall in temperature followed by a rise, and the death of some animals. Table 2 shows the results of the measurements of the

TABLE 2

Action of juice of non-fatigued muscles on body temperature

NUMBER OF EXPERIMENT	INITIAL TEMPERATURE	FALL OF TEMPERATURE AFTER INJECTION, 15 MINUTE INTERVALS										MAXIMUM FALL
		15	30	45	1 hour	15	30	45	2 hours	15	30	
	°C.											
19	38.7	0.8	0.9	0.6	0.6	0.5	0.5	0.6	0.6	0.5	0.4	0.9
20	39.5	2.4	1.9	1.8	1.8	1.3	1.1	0.8	0.4	0.7		2.4
21	39.2	2.2	1.8	1.6	1.2	1.0	0.5	0.6	0.6	0.3		2.2
23	38.3	0.9	1.1	1.4	1.3	1.0	1.1	1.1	1.3	1.1	0.6	1.4
24	37.9	1.5	2.2	1.7	1.2	1.3	0.9	0.8	0.6	0.6	0.3	2.2

temperatures in the five most complete experiments, cats here serving as the source of the muscle juice and guinea pigs as the test animals. It is noticeable here that the maximum fall of temperature is, in general, even greater than with the fatigue juice, the average being 1.8°C. and the maximum 2.4°.

Of the eight animals injected with non-fatigue juice, four died. Of these one, injected by way of the external jugular vein, died immediately afterward, with symptoms of an embolus; two died on the day following the intraperitoneal injection, and one three days afterward. The autopsies in all cases showed conditions not differing from those dead after the intraperitoneal receipt of fatigue juice.

Our observations on the action of muscle juice on animals thus fail to reveal in the juice of fatigued muscles the presence of anything acutely toxic and peculiar only to fatigue. They do justify the assumption, however, that a generally depressing substance is present in both the fatigue and the non-fatigue juice. The continual and fatiguing work of

the animals in the wheel, their excessively rapid respiration and quiet state at the end, and the quick oncoming of rigor after death, are all conceivably explicable on the supposition that in the process of fatigue the muscles received too little oxygen and manufactured lactic acid. The treatment of both the fatigued and the control muscle in the preparation of the juice would only add to the stock of acid in the former and lead to its formation in the latter. It would therefore be expected that both preparations would contain in the end a considerable quantity of acid. Whether the acid alone is responsible for the depression we cannot say, but it is clear that if any single chemical substance is the causative agent it is present in both the fatigue and the non-fatigue juice.

The action of muscle juice on muscles. In an endeavor to demonstrate the action of his supposed "fatigue toxin" on muscles Weichardt performed a number of experiments on mice in which he recorded graphically the contractions of the gastrocnemius muscle in situ. The muscle was stimulated by brief faradic currents every second until exhausted. "Normal curves" were obtained from normal animals; "toxin curves" from other animals which had received a dose of "fatigue toxin" injected into the peritoneal cavity. No mention is made of the way in which such toxin was prepared or of the number of animals of each class used. The published curves show less work performed by the intoxicated individuals, but no computations of the actual amounts of work performed by either class are presented.

We have performed a series of experiments on the action of the muscle juice of both fatigued and non-fatigued animals on muscles. The juice was prepared as already stated, cats being used always as its source. In each test a frog was killed, and its two gastrocnemius muscles were excised, attached to muscle levers, loaded with equal weights, and prepared for simultaneous stimulation by the same single induction shocks and for the recording of the contractions as vertical lines on a slow drum. The skin was left about each muscle and was tied about the tendo Achillis below, thus forming a bag about the muscle, open at the top. The bag surrounding one of the muscles was filled by the muscle juice, which thus continually bathed the muscle tissue. The other muscle received no juice and served for control. The stimulation then proceeded until the two muscles were exhausted. The duration of the work of each muscle was noted, and from the graphic records the total work done by each was computed. The following table 3 shows the effect of the fatigue juice.

It is here seen that with the normal muscle the average duration of the working power was 43.2 minutes and the average amount of work performed 21,039 gram millimeters. For the muscle under the influence of the fatigue juice the average duration of working power was 19.8

minutes, a decrease of 54 per cent, and the average amount of work performed was 10,082 gram millimeters, a decrease of 52 per cent. The depressing action of the fatigue juice is thus very striking.

Before assuming that this result is due to a fatigue substance the above figures should be compared with those of a similar series of experiments shown below, table 4, similarly performed, but in which the action of the juice of non-fatigued muscles was studied.

TABLE 3

Action of juice of fatigued muscles on muscle

NUMBER OF EXPERIMENT	CONTROL GASTROCNEMIUS		GASTROCNEMIUS + FATIGUE JUICE	
	Duration of work in minutes	Work done in gm. mm.	Duration of work in minutes	Work done in gm. mm.
1	90	43,298	33	19,640
2	36	16,560	19	7,695
3	29	12,148	10	3,066
4	23	11,003	12	4,685
5	38	22,189	25	15,327
Average.....	43.2	21,039	19.8	10,082
Percentage.....	100	100	46	48

TABLE 4

Action of juice of non-fatigued muscles on muscle

NUMBER OF EXPERIMENT	CONTROL GASTROCNEMIUS		GASTROCNEMIUS + NON-FATIGUE JUICE	
	Duration of work in minutes	Work done in gm. mm.	Duration of work in minutes	Work done in gm. mm.
6	43	23,030	15	6,271
7	49	22,854	23	13,844
8	46	18,748	20	6,938
9	39	17,023	18	9,227
10	39	20,810	18	11,641
11	33	8,492	17	6,946
Average.....	41.5	18,492	18.5	9,144
Percentage.....	100	100	44	49

Here the surprising fact appears that, while the normal muscle continues to contract approximately as long and to perform approximately as much work as the normal muscle in the previous series—the slight differences being of no significance—the muscle under the influence of the non-fatigue juice loses approximately the same amount as before, namely, 56 per cent in working time and 51 per cent in total amount of work done.

The hypothesis of an acutely toxic substance, present in the fatigue and absent in the non-fatigue juice, is thus no more justified here than in the experiments with the whole animal.

Weichardt's claims may be criticized adversely from several points of view.

In his search for a "fatigue toxin" through the use of animals Weichardt emphasizes the necessity of carrying the fatigue process to an extreme end, complete exhaustion and even death. He thus incurs the criticism of the physiologist, for he demands an artificial condition which is rarely realized in nature—putting an animal to death by overwork is changing a physiological into a pathological phenomenon. But if fatigue be due to the action of a specific "fatigue toxin," then the toxin should be present whenever fatigue symptoms are present, that is, at all times after considerable work, and it ought to be capable of demonstration without the over-fatigue which Weichardt practises. One cannot help suspecting that in his exhausted animals chemical changes have taken place which do not occur in normal fatigue. Moreover, the physiologist may rightly claim that stupor, lowering of the body temperature and slowing of the respiration are hardly adequate criteria of the existence of normal fatigue.

It is only in his earlier work, however, that Weichardt relied upon the fatigue of animals for the production of his supposed toxin. He soon found its chemical preparation more satisfactory. But in reading the accounts of his chemical methods it is difficult to learn exactly what their author was dealing with. It is hardly conceivable that oxidation, reduction and hydrolysis, as employed by him with solutions of protein, would yield chemically identical substances. It is, moreover, not clear whether his products are single chemical substances or mixtures of chemical substances. It is not clear whether he regards all of his so-called "Ermüdungsstoffe," obtained from whatever source, physiological or diversely chemical, as one and the same substance—"toxin" and "toxins" are used indiscriminately in his writings.

Although Weichardt's experiments seem to have been numerous, accounts of them are confined largely to accounts of single selected experiments, and the reader is not offered sufficient information to enable him to form a judgment of the degree of statistical support which his work gives to his conclusions. There is evident, too, a proneness to accept without question favorable evidence, however intrinsically strange. A striking instance of this, which is quoted approvingly by Weichardt, is supplied by Lorentz (6) in his report of the reputed effects of the supposed "fatigue antitoxin" received by inhalation. The rapidity and accuracy of the mental work of certain school pupils were tested by arithmetical problems before and after a five-hour period of instruction. The degree of fatigue resulting was thus measured. After this was es-

tablished the air of the school room after a morning test was liberally sprayed with a 1 per cent solution of antikenotoxin. The afternoon test then showed that the rapidity of computation was increased by 50 per cent; the number of errors had decreased; and some pupils who were usually sleepy and tired at the end of the period of instruction had a higher grade after than before the five-hours' period! It is not surprising that in an extensive investigation under similar conditions Konrich (7) was wholly unable to confirm these results.

It is conceivable that by Weichardt's extreme treatment of animals and his unique ways of treating proteins outside animal bodies he obtained substances or mixtures of substances that are toxic to the living organism, but he is not justified, we believe, in associating any one of such substances in a specific causal relation with the physical phenomenon of normal fatigue.

SUMMARY

1. When juice is pressed out of the muscles of fatigued animals and injected into other animals there result stupor, no constant effect on respiration, a temporary lowering of the body temperature, and occasionally subsequent death.

2. Such fatigue juice when administered to excised muscles causes a marked decrease in their working power, as shown by a shortening of the working period and a decrease of the total work performed.

3. Juice from the muscles of non-fatigued animals exerts the same action as fatigue juice, both on animals and on excised muscles.

4. The experiments thus fail to confirm Weichardt's assumption of the existence of a specific toxin of fatigue.

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ADDITIONAL DATA CONCERNING WEICHARDT'S SUPPOSED "FATIGUE TOXIN"

FREDERIC S. LEE

From the Department of Physiology, Columbia University, New York

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Aronovitch and I (1) were unable to confirm Weichardt's claim of the existence of a specific "fatigue toxin." But in our experiments cats, because of their ready fatiguability in our fatigue wheel, were used as the source of the muscle juice, and guinea pigs, because of their docility, were used as the test animals. Thus, by injecting into one species of animal muscle juice obtained from a different species, we incurred the possible criticism that we were merely demonstrating the well-known action of a foreign protein. To obviate this objection, I have performed additional experiments in which guinea pigs were used for both purposes.

The method employed was, in general, similar to that used by Aronovitch and myself, and consisted in thoroughly fatiguing animals, killing them, squeezing the juice from their muscles, and injecting it into fresh animals. For fatiguing the guinea pigs I made use of the treadmill which Hastings (2) had found effective with dogs, and has since been employed by others in this laboratory. It consists of a running-track caged in and propelled by an electric motor at a speed which can be varied according to the running capacity of the individual animal. The guinea pigs, either with or without a few minutes' preliminary training, usually adapted themselves readily to a rate of approximately three-quarters of a mile per hour and were able usually to continue running for more than three hours. In the latter part of the period fatigue became more and more evident, and when the animal ceased to run further, it was removed and immediately killed by decapitation. The blood flowed freely from the vessels and a sample was collected for the determination of its sugar content. The results of such determination will be presented elsewhere. The skeletal muscles were electrically stimulated en masse until they ceased to contract. They were then cut away, freed from connective and fatty tissue, and ground thoroughly in a mortar into a pulp. This was wrapped in cheese-cloth and submitted to very heavy pressure in a strong steel vise. As much juice as possible was thus squeezed out, the amount from a single animal averaging 24 cc. This juice, which was always strongly acid to litmus, was filtered and warmed to body temperature, and a measured quantity of it, usually 10

cc., was injected intraperitoneally into a fresh guinea pig. The effects produced by this injection were then observed. Eight experiments of this kind were performed.

The action of muscle juice on homologous species of animals. Following Weichardt's procedure and as in the experiments with Aronovitch my attention was directed, after injection, to three phenomena: The general behavior of the animal, with especial reference to possible stupor, the rate of respiration, and the body temperature; and these were observed at fifteen-minute intervals during a period of two to six hours. The condition of the animal during several days subsequent to the injection was also studied.

While it can hardly be said that pronounced stupor followed the injection, the animal usually became quiet and mildly soporific, allowing itself to be handled without resistance. In this respect, its behavior was not fundamentally different from that of the animals in the series with Aronovitch, although the soporific effect in the new series was perhaps somewhat less in degree.

As in the series with Aronovitch, the rate of respiration was not affected by the injection in any constant manner. There was much irregularity in the rate, as is not unusual in normal guinea pigs.

The most pronounced and constant effect of the injection was that on the temperature of the body. After the receipt of the muscle juice the temperature, as measured in the rectum, began immediately to fall. The lowest point was reached within a period varying from thirty minutes to two hours, after which the temperature more gradually rose to, or nearly to, the initial point during the period of observation. Of the eight experiments, one, number 66, proved an exception to the rule in showing only minute temperature changes ranging from a preliminary rise of 0.3° to a later fall of 0.2°. Table 1 presents the details of all experiments except number 66, and figure 1 represents the curve of experiment 34, a typical experiment. It may be added incidentally that of the seven animals two died within twenty-four hours and one three days after the injection, while the remaining four continued in good health.

While the effect here is, in general, similar to that in the former series in which non-homologous animals were used, there appear two differences: a much greater average fall of temperature and a slower average recovery. Thus, the possible objection that in the former series the results might have been due to the action of a foreign protein is at once ruled out—whether the muscle juice comes from a different or the same species the sequence of its administration is an immediate fall of body temperature followed by a gradual recovery.

Are we then demonstrating the action of a fatigue toxin? Here again a control series of experiments supplies the answer—a series in which the procedure was in all respects the same as with the fatigue juice save that the

guinea pigs supplying the juice were not fatigued at all. Eight such experiments were performed. There resulted quiet and occasionally real stupor; no constant change in respiration; an immediate fall of body temperature followed by gradual recovery; and the death within twenty-four hours of six of the animals.

The temperature data of seven of these experiments are presented in table 2 and the curve of experiment 53 is plotted as figure 2. Experiment

TABLE 1

Action of juice of fatigued muscles on body temperature of homologous species

NUMBER OF EXPERIMENT	INITIAL TEMPERATURE	FALL OF TEMPERATURE AFTER INJECTION, 15 MINUTE INTERVALS																						MAXIMUM FALL
		15	30	45	1 hour	15	30	45	2 hours	15	30	45	3 hours	15	30	45	4 hours	15	30	45	5 hours			
	°C.																							
32	37.7	1.1	1.3	1.6	0.9	0.3	0																	1.6
33	39.3	2.7	3.3	3.0	2.3	1.9	1.4	1.2	1.0	1.1	0.5	0.5	0.1	0										3.3
34	39.4	1.5	2.2	2.2	2.1	1.9	1.5	1.4	0.8	0.7	0.6	0.3	0.2	0.4	0.4	0.3	0.1	0.1						2.2
36	39.4	1.3	1.7	1.6	1.8	1.8	2.1	2.2	2.4	2.2	2.1	2.0	1.8	1.7	1.7	1.4	1.3	1.2	1.0	0.7	0.7			2.4
62	39.4	0.2	0.4	+0.1	0.2	0.1	0.2	0.6	0.4	0.5	0.2	0.5	0.6											0.6
64	39.8	0.5	1.2	1.6	2.1	2.2	2.3	2.4	2.6	2.5	2.5	2.3	2.3	2.1	2.0	2.0	2.1	2.0	2.0	1.8	2.0			2.6
67	38.9	1.4	2.3	2.5	2.7	3.0	3.3	3.3	3.3	3.0	2.8	2.6	2.4	2.2	2.1	1.9	1.5	1.1	1.1	0.6	0.3	+0.3		3.3

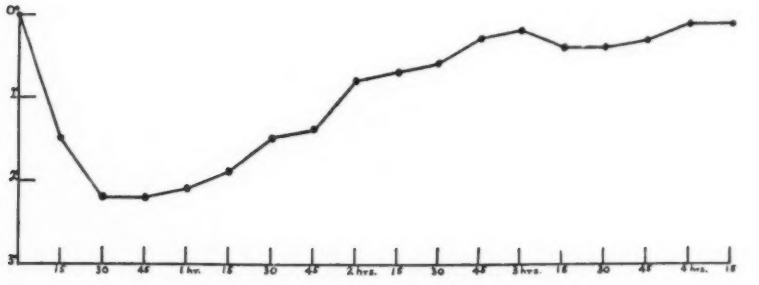

Fig. 1. Action of juice of fatigued muscles on body temperature. Experiment 34, table 1.

38 showed a fall of temperature so far in excess of all the others—more than five degrees and so far beyond the minimum limit of the clinical thermometer as to be incapable of measurement—that it has been omitted from the published records.

Here again the tale is repeated: as in the experiments with Aronovitch non-fatigue juice produces the same effect as fatigue juice. This is true whether the recipient of the juice is of the same species of animal as the donor, or of a different species.

Enough evidence has now been presented, I believe, to make it clear that Weichardt's assumption of the existence of a specific toxin of fatigue quite lacks a reliable experimental basis and is quite needless to account for the well-known facts of fatigue.

The action of lactic acid on animals. Since the muscle juice in both the above series was always strongly acid to litmus, I was curious to know whether its physiological action might be due to its acid content. This question had been raised in the paper by Aronovitch and myself, but we did

TABLE 2

Action of juice of non-fatigued muscles on body temperature of homologous species

NUMBER OF EXPERIMENT	INITIAL TEMPERATURE	FALL OF TEMPERATURE AFTER INJECTION, 15 MINUTE INTERVALS																				MAXIMUM FALL
		15	30	45	1 hour	15	30	45	2 hours	15	30	45	3 hours	15	30	45	4 hours	15	30	45	5 hours	
	°C.																					
35	38.8	1.1	1.2	1.4	1.3	1.0	0.8	0.9	1.0	1.0	0.9	0.9	0.5	0.6	0.7	0.4	0.4	0.2	0.3	0.2	0.2	1.4
37	39.3	0.8	0.9	0.8	1.0	1.1	1.3	1.2	1.4	1.2	1.0	0.8	0.7	0.6	0.5	0.6	0.5	0.5				1.4
39	39.8	1.1	1.4	1.4	1.0	1.0	0.8	0.5	0.6	0.9	1.1	1.2	1.2	0.8	0.8	0.4	0.4	0.2	0.2	0.2	0.5	1.4
51	38.9	0.3	1.4	1.5	1.7	1.5	1.5	1.3	1.3	1.3	1.5	1.2	0.9	1.1	0.7	0.7	0.9	0.4	0.3	0.3	0.3	1.7
53	38.0	1.2	1.6	2.2	2.2	1.8	1.0	0.6	0.3	0.1	0											2.2
59	39.6	1.1	1.8	2.3	2.8	3.2	3.3	3.1	3.1	3.0	2.8	2.3	2.1	1.8	1.5	1.3	1.1	1.0	0.9	0.8	0.8	3.3
60	39.1	0.9	1.2	1.8	2.0	1.9	2.1	2.2	2.4	2.4	2.4	2.3	2.2	2.4	2.3	2.1	2.0	1.9	2.0	1.9	1.7	2.4

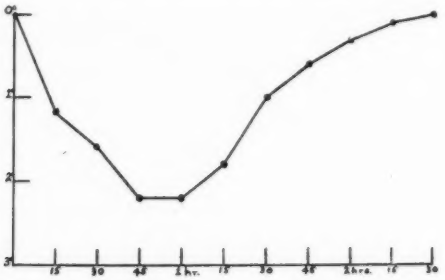

Fig. 2. Action of juice of non-fatigued muscles on body temperature. Experiment 53, table 2.

not attempt to answer it experimentally. I have therefore made a few experiments bearing upon it.

Dr. C. D. Murray courteously determined the pH in two specimens of juice, one from non-fatigued and one from fatigued guinea pigs, and found that of the former to be 5.6 and that of the latter 5.8. Through the courtesy of Dr. F. B. Flinn, determinations of lactic acid were made in four specimens each of non-fatigue and fatigue juice. The figures, expressed in milligrams of lactic acid in 100 cc. of juice, were: for the non-

fatigued animals, 222, 198, 212, 210, the average being 210; and for the fatigued animals, 223, 207, 197, 247, the average being 218.

Seven healthy, non-fatigued guinea pigs were injected intraperitoneally, each with 10 cc. of a solution of lactic acid consisting of 220 mgm. in 100 cc. of an 0.9 per cent solution of sodium chloride and warmed to body temperature. Following the injection the animals became very quiet, sometimes relaxed muscularly, sometimes trembling as if feeling a chill; there was no observable effect on respiration; there were no deaths. But there was an

TABLE 3

Action of lactic acid on body temperature

NUMBER OF EXPERIMENT	INITIAL TEMPERATURE	FALL OF TEMPERATURE AFTER INJECTION, 15 MINUTE INTERVALS												MAXIMUM FALL
		15	30	45	1 hour	15	30	45	2 hours	15	30	45	3 hours	
	°C.													
41	39	0.7	0.8	0.8	0.5	0								0.8
42	38.7	1.1	1.7	1.5	1.2	1.1	0.7	0.2	0					1.7
49	39.2	1.0	1.1	1.2	1.2	1.3	0.9	0.7	0.5	0.5	0.4	0.2	0	1.3
52	38.3	0.1	0.5	0.6	0.4	0.4	0.3	0.1	0					0.6
56	39.2	0.1	0.4	0.2	0.2	0.1	0							0.4
57	39.5	0.6	0.5	0.7	0.7	0.5	0.3	0.4	0.3	0.3	0.5	0.2	0.4	0.7
58	39.7	0.8	0.9	1.6	1.4	1.2	1.0	0.7	0.6	0.6	0.1	0		1.6

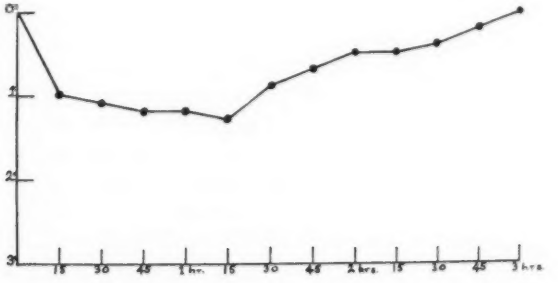

Fig. 3. Action of lactic acid on body temperature. Experiment 49, table 3.

immediate fall in body temperature, followed by gradual recovery. The data on body temperature are presented in table 3 and the curve of the typical experiment 49 is plotted in figure 3.

Still again we see presented the familiar picture. The effect is not so profound as with the muscle juice, but it is of the same sort. It may therefore reasonably be inferred that the temperature-depressing action of muscle juice, whether from fatigued or non-fatigued animals, is due in part at least to their content of lactic acid.

SUMMARY

1. When juice is pressed out of the muscles of fatigued guinea pigs and injected into fresh guinea pigs there result quiet, no constant effect on respiration, a temporary lowering of the body temperature and occasionally subsequent death.

2. Juice from the muscles of non-fatigued guinea pigs exerts the same action on fresh guinea pigs as fatigue juice.

3. Lactic acid, when injected into guinea pigs, is followed by quiet and a temporary fall of body temperature, which is less in extent than that following the injection of muscle juice.

4. The experiments fail to confirm Weichardt's assumption of the existence of a specific toxin of fatigue.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

Influence of Experimental Changes in Blood Sugar Level on Gastric Hunger Contractions

E. BULATAO[1] AND A. J. CARLSON

From the Hull Physiological Laboratory of the University of Chicago

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In the conditions so far found to increase gastric hunger contractions there is either a decrease in tissue glycogen (starvation, muscular exercise, ice-pack, phlorizin), or inability of the tissues to burn sugar (pancreatic diabetes). Furthermore, one of the symptoms accompanying insulin hypoglycemia in man is increased hunger. These observations point to the possibility that the availability of carbohydrates for utilization by the stomach motor tissues is an important factor in the genesis or intensity of the gastric hunger contractions. If this hypothesis is correct, experimental increase in the level of blood glucose in normal animals should produce inhibition of hunger contractions, and experimental decrease in that level should lead either to onset of hunger contractions in the quiescent stomach, or to augmentation of hunger contractions when these are already present. It seems equally clear that increase in the blood sugar in completely pancreatectomized animals should not inhibit the gastric hunger contractions. But in animals that have been rendered completely diabetic administration of insulin should cause a temporary depression or inhibition of the hunger contractions, followed by augmentation of the contractions when the insulin has induced a certain degree of hypoglycemia. This secondary augmentation of the gastric contractions in insulin hypoglycemia of the diabetic animal should, in turn, be inhibited by the intravenous administration of glucose, as long as the insulin is present in the body. All of these conjectures can be answered by direct experiment, and positive results in all cases would greatly enhance the probability of the hypothesis that the type of metabolism (increased oxidation of lipoids) in the gastric motor tissues is a factor in the initiation and amplitude of the gastric hunger contractions.

Methods. Gentle dogs with gastric fistulae, produced according to the technique described by one of us, were used. The gastric movements were recorded by means of the balloon and water manometer. Quantitative

[1] Fellow, Rockefeller Foundation, from the University of the Philippines.

determination of blood sugar was made by the Folin-Wu method. The dogs were trained to lie down quietly during the 2 to 4 hour experiments. In most cases the experiments were performed 20 to 48 hours after the last meal.

Experimental hyperglycemia was produced by intravenous injection of 50 per cent solution of glucose; and hypoglycemia, by subcutaneous administration of insulin. We used the Eli Lilly & Co. "iletin" and an insulin prepared in our laboratory by Mr. Shaw. Records were made of normal gastric hunger contractions for two to four hours on days when no experiment was performed to determine the types of contractions and their periodicity characteristic of the individual.

In one of the dogs with a healed gastrostomy the pancreas was extirpated, the completeness of the extirpation being confirmed by post-mortem examination.

The glucose used for the intravenous injections was of Merck's and Pfannenstiel's manufacture.

RESULTS. *1.* Two to four hour records of hunger contractions showed the following individual characteristics: Dog I. Type I contractions, hunger periods lasting 50 to 60 minutes, and quiescent periods of 5 to 20 minutes; dog II, types I and II contractions without either quiescent or tetany periods; dog III, mostly type II contractions, no quiescent periods and only occasional type III contractions (gastric tetany) lasting 1 to 2 minutes. Dog IV, types I and II hunger contractions before, and types II and III hunger contractions after removal of the pancreas. Quiescence of the stomach could therefore be interpreted as inhibition in dog I only when it occurred in the middle of a hunger contraction period.

2. In the normal dogs (10 to 15 kilo body weight) *inhibition of gastric hunger contractions and gastric tonus always followed the injections of 5 to 10 grams (50 per cent solution) of glucose* (fig. 1). This inhibition, lasting for 10 to 60 minutes, is frequently not complete. The return to normal gastric tonus and hunger contractions is gradual.

This glucose inhibition of the gastric hunger contractions is not due to the hypertonicity of the 50 per cent solution because the injection of the same volume of equimolecular solution of sodium chloride (16 per cent) is not followed by inhibition beyond slight decrease in amplitude of the contractions attributable to the handling of the dog. Similarly, intravenous injections of 5 to 10 grams of a 50 per cent solution of lactose have no effect on the gastric hunger contractions (fig. 2). It would therefore seem that it is the hyperglycemia as such which produces the gastric inhibition in normal dogs.

3. Gastric hypertonus and hypermotility following insulin administration in normal dogs. The increased gastric motility after subcutaneous injection of 20 to 40 units of insulin is as definite as the gastric inhibition in

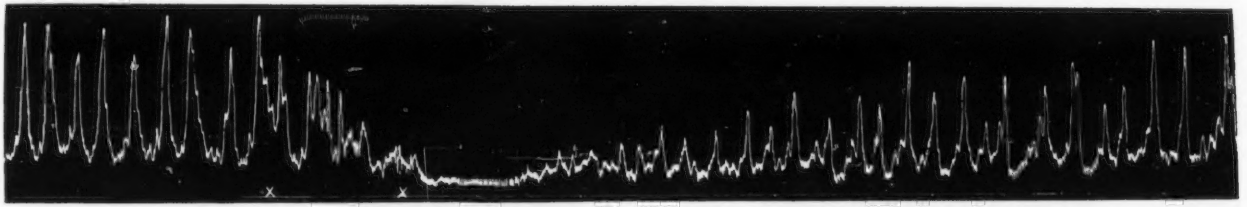

Fig. 1. Dog. Water manometer tracing of the gastric hunger contractions. *x-x'*, intravenous injection of 10 grams of glucose (50 per cent solution), showing inhibition of the gastric tonus and hunger contractions by hyperglycemia. (Total time of record, 60 minutes.)

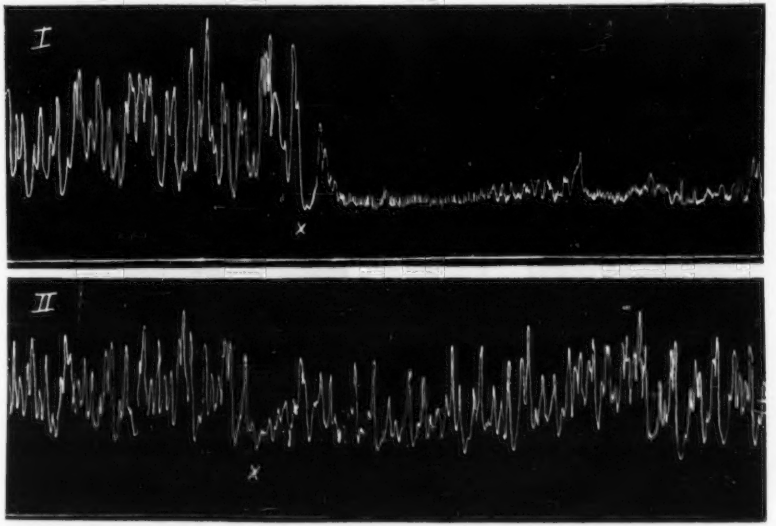

Fig. 2. Dog. Water manometer tracings of gastric hunger contractions. *I*, *x*, intravenous injection of 10 grams of glucose. *II*, *x*, intravenous injection of 10 grams of lactose. Showing absence of inhibition from lactose hyperglycemia.

normal dogs produced by glucose. Increase in the gastric tonus and in height and frequency of contractions usually appears in 50 minutes (minimum 10, maximum 60), gradually developing into gastric tetany in one hour (minimum 40, maximum 80 minutes) after the injection. The gastric

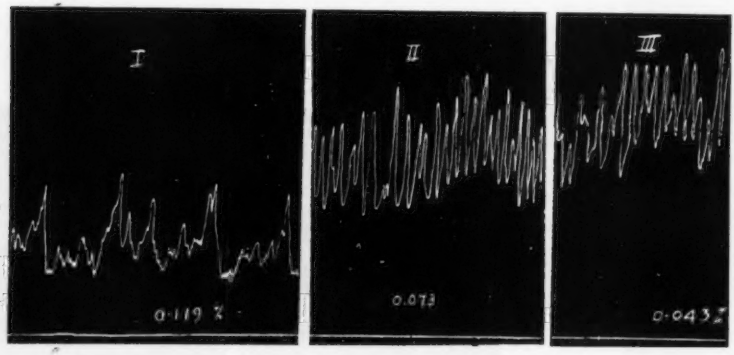

Fig. 3. Dog. Water manometer tracings of the gastric hunger contractions. *I*, before injection of insulin. Blood sugar 0.119. *Ii*, 60 minutes after injection of 40 units insulin. Blood sugar, 0.073. *III*, 120 minutes after the insulin injection. Blood sugar, 0.043. Showing the augmentation of gastric hunger contractions and tonus parallel with insulin hypoglycemia in normal dogs.

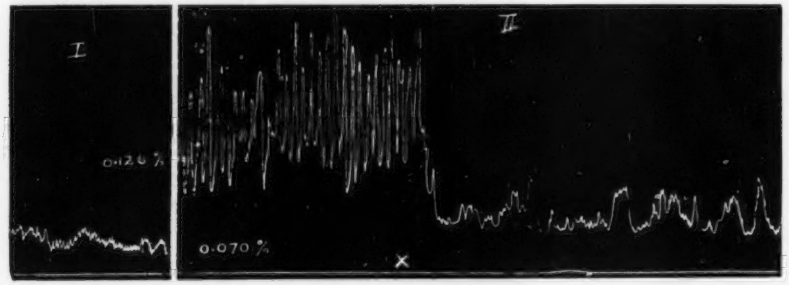

Fig. 4. Dog. Water manometer tracing of the gastric hunger contractions. *I*, before insulin injections. Blood sugar, 0.126. *II*, 90 minutes after injection of 40 units of insulin. Blood sugar, 0.070. *x*, intravenous injection of 10 grams of glucose. Showing initiation of gastric tetany parallel with insulin hypoglycemia, and inhibition of this tetany by glucose.

tonus may rise from 1 to 4 cm. water, in the control, to 6 to 15 cm. water during the gastric tetany (figs. 3, 4, 5). The stomach remains in incomplete tetany until glucose is injected, or until the dog goes into hypoglycemia, restlessness, spasms and convulsions. During the convulsive period

there is alternate tetany and atony of the stomach, the latter condition predominating, probably due to reflex inhibition of the stomach.

4. The effect of glucose on gastric tetany of hypoglycemia. On intravenous injection of 5 to 10 grams of glucose (50 per cent solution), there is almost always a total abolition of hunger contractions lasting 5 to 15 minutes. In cases when the inhibition is not complete, the hunger contractions are feeble and far apart. The gastric tonus falls. The return of gastric tonus and hunger contractions is gradual and in 30 to 90 minutes the stomach may again go into the tetany of hypoglycemia if a large dose of insulin has been administered (figs. 4, 5).

Intravenous injections of 10 to 20 grams of lactose (50 per cent solution) do not inhibit the gastric tetany of hypoglycemia.

5. The effect of glucose and insulin on the gastric hunger contractions in diabetic dogs (complete pancreatectomy). a. In a diabetic dog, weighing 13 kilograms, before pancreatectomy, intravenous injection of 5, 10 or 20 grams of glucose (50 per cent solution) did not produce the typical prolonged inhibition of the gastric hunger contractions seen in normal dogs (fig. 6). The injections usually induce slight and temporary inhibitions (1 to 3 minutes). These are probably reflex inhibitions induced by the injection process, rather than due to additional hyperglycemia.

b. When 20 to 40 units of insulin were injected hypodermically into the diabetic dog showing the hypermotility of the empty stomach typical for the condition of absolute diabetes, there was at first no influence on the gastric tonus and hunger contractions. Fifteen to 20 minutes after the insulin injection the gastric tonus began to decrease, and the hunger contractions became gradually weaker with longer intervals of rest, until at 30 to 60 minutes following the insulin, the stomach became relatively atonic and quiescent. This quiescence may last for 10 to 20 minutes, when the hunger contractions and gastric tonus return gradually, and the typical gastric tetany of hypoglycemia appears 60 to 80 minutes after insulin administration, when the blood sugar has fallen to 0.08 to 0.07 per cent. At this stage the gastric tonus and hunger contractions are usually much greater than before the insulin administration. If 10 to 20 grams of glucose are injected intravenously at this stage, one obtains the same inhibition of gastric tonus and hunger contractions seen in normal animals (fig. 7).

It is thus clear that glucose and insulin have initial effects of the opposite character in the normal and the diabetic dog. In the normal dog insulin induces gastric hypertonicity and gastric tetany (at the 0.08 to 0.07 blood sugar level) without primary inhibition. In the diabetic dog the primary action of insulin on the gastric tonus and hunger contractions is inhibition and the gastric tetany of hypoglycemia comes later. Glucose inhibits the gastric tonus and hunger contractions in normal dogs, but not in diabetic dogs, when not under the influence of insulin.

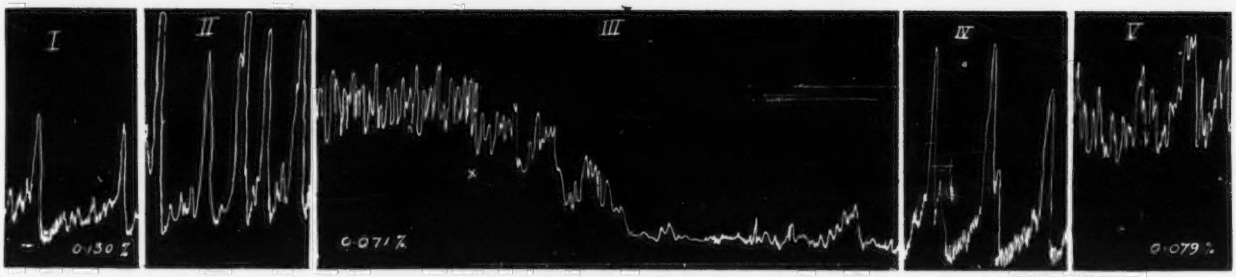

Fig. 5. Dog. Water manometer tracings of the gastric hunger contractions. *I*, before insulin. Blood sugar, 0.113. *II*, 40 minutes after injection of 50 units insulin. *III*, 90 minutes after the insulin injection. Blood sugar, 0.071. Stomach in complete tetanus. *x*, intravenous injection of 20 grams glucose. *IV*, 60 minutes after the glucose injection. *V*, 90 minutes after the glucose injection, blood sugar, 0.079. Showing gastric hypertonus and hypermotility parallel with insulin hypoglycemia, and inhibition of the gastric hypertonus by glucose.

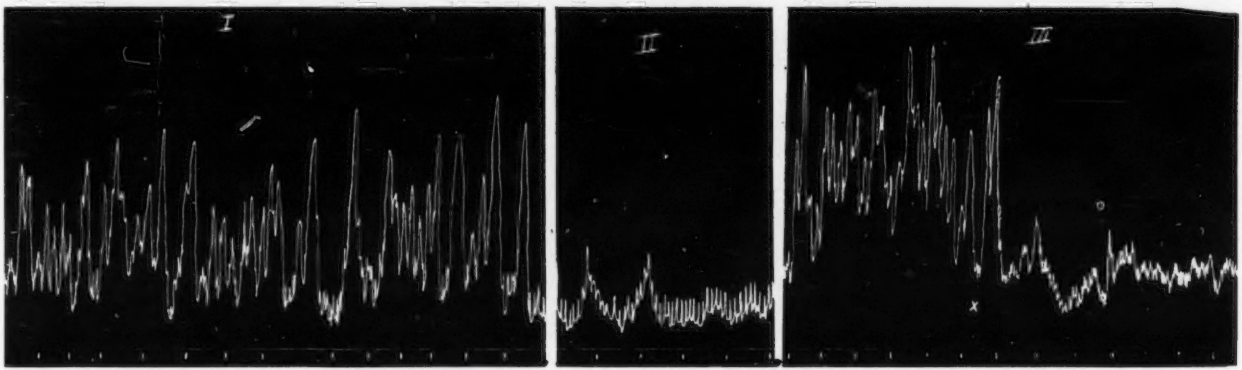

Fig. 7. Diabetic dog, 21 days after pancreatectomy. Water manometer tracing of the gastric hunger contractions. *I*, before insulin injection. *II*, 35 minutes after injection of 40 units of insulin. *III*, 120 minutes after the insulin injections. *x*, intravenous injection of 10 grams of glucose. Showing initial inhibition by insulin of the gastric hunger contractions in diabetic dogs, the iniation of gastric hypertonus parallel with the insulin hypoglycemia, and the inhibition of this hypertonus by glucose. Time, minutes.

DISCUSSION. We know that raising the blood sugar level of normal animals leads to an increase in the glycogen content of the tissues, and there is some evidence that the same thing takes place in the diabetic animal when insulin is administered. We also know that, when available, glycogen or glucose is the first source of energy in tissue activity. According to Woodyatt, forced intravenous injection of glucose leads to increased sugar oxidation. We therefore advance, as a working hypothesis, that the influence on gastric motility of hypoglycemia, and of intravenous administration of glucose in normal and diabetic dogs (with and without insulin) is due to change in the energy metabolism of the gastric motor tissues when the available glycogen is reduced or sugar cannot be oxidized. So far, the only known facts that do not clearly fit this hypothesis are the

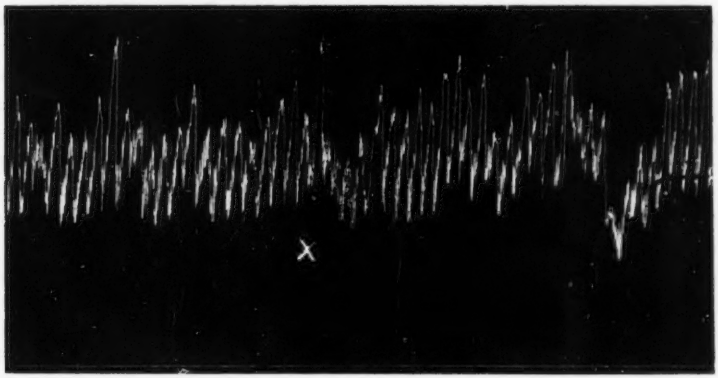

Fig. 6. Dog, 15 days after complete pancreatectomy. Water manometer tracing, showing gastric hypertonus and hunger contractions. *x*, intravenous injection of 10 grams of glucose, showing failure of glucose to inhibit the gastric tonus and hunger contractions in diabetic dogs.

augmentation of the gastric tonus and hunger contractions by transfusion of blood from starving and from diabetic dogs into normal dogs, as reported by Luckhardt and Carlson. This would seem to point to the presence in starvation and diabetic bloods of some substance that stimulates the gastric hunger mechanism. In this connection we may refer to a series of studies from Professor Magnus' laboratory by Wieland, Heux, v. Kühlemein and Arai, which attempt to demonstrate that cholin is a motor hormone of the gut, acting on Auerbach's plexus. It is possible that the gastric motor tissues, when they oxidize lipoids in conditions of reduced glycogen, liberate cholin, which in turn may stimulate the gastric motor mechanism. This possibility is under investigation. But so far no studies have been

reported on the influence of cholin on tonus and contractions of the empty stomach (hunger mechanism), and our own preliminary experiments with chloramine and cholin chloride have produced only inhibition of the empty stomach.

We are, of course, aware of the probable complexity in the blood and tissue changes accompanying hypo- and hyperglycemia, besides the tissue glycogen, initial acidosis and the energy metabolism factors. Our experiments so far are not simple, and we must reckon with other possible interpretations than those proposed here. The motor nerves to the stomach may be involved. Cannon has reported that insulin hypoglycemia leads to an increased output of adrenalin, sufficient to accelerate the "denervated heart." We have not considered that excess adrenalin output is a factor in the genesis of the gastric tetany accompanying hypoglycemia, because on intravenous administration of adrenalin we have never obtained any other effect than inhibition of the gastric hunger mechanism. But we admit the possibility that such artificial administration of a commercial product may not be identical in rate and character with an augmented secretion of the adrenal medulla.

Our results explain the excess hunger felt by patients as one of the early symptoms of hypoglycemia following large doses of insulin. This hunger parallels the increased gastric tonus and contractions. Our hypothesis would also explain the rapid suppression of hunger in fasting persons on taking sugar by mouth, as well as the depression of hunger, especially in children, on eating candies and other sweets between meals.

SUMMARY

1. Experimental hyperglycemia produced by intravenous injection of glucose inhibits normal gastric hunger contractions. This effect is not due to the hypertonicity of the injected solutions since similar injection of lactose, or sodium chloride does not have this effect.

2. In insulin hypoglycemia (normal dogs) increase in gastric tonus and hunger contractions (tetany) appears at a blood sugar concentration of 0.08 to 0.07 per cent. As the blood sugar falls toward the convulsion level, the stomach motor mechanism usually shows alternate periods of atony and tetany, the inhibition predominating. Prior to the gastric tetany of hypoglycemia, the gastric hunger contractions are more frequent and usually slightly stronger.

3. Glucose inhibits the gastric tetany of hypoglycemia. Lactose does not produce this effect.

4. In diabetic dogs insulin produces a primary depression of gastric tonus and contractions, followed by increased gastric tonus and contractions when the initial stage of hypoglycemia is reached.

5. Intravenous injections of glucose do not inhibit gastric tonus and hunger contractions in diabetic dogs except when hypoglycemia and gastric tetany are induced with insulin.

6. It is suggested that under ordinary conditions gastric tonus and contractions (empty stomach) increase parallel with the reduction of the tissue glycogen, and an enforced energy metabolism of lipoids on the part of the motor tissues of the stomach.

We are under obligations to the Eli-Lilly Company for supplying, free of charge, part of the insulin used in this investigation.

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THE QUESTION OF FAT ABSORPTION FROM THE MAMMALIAN STOMACH

TAKEO INOUYE[1]

From the Laboratory of Physiological Chemistry, Yale University, New Haven, Connecticut

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The question whether fat can be absorbed directly from the mammalian stomach has often been discussed both from the chemical and the histological standpoints. The chemical evidences offered first by Klemperer and Scheurlen (1) in 1889 and confirmed again by Mendel and Baumann (6) in 1915 in a more satisfactory way, favored the assumption that fat is not absorbed from the stomach, in harmony with the current view regarding the gastric functions. However a number of investigators—Kischensky (2), Lamb (3), Weiss (4), Greene and Skaer (5), and more recently Hirayama (7)—who have made the histological observations on different kinds of animals—kittens, puppies, cats and rats—after feeding fat, have concluded that ingested fat does pass through the stomach wall because in their studies fat droplets appeared to be present in the stomach mucosa. Greene and Hirayama have reported the stomach mucosa to be filled abundantly with fat globules after fat feeding, in adult dogs as well as in youngs ones, and have pointed out that the number of these droplets bears a proportional relationship to the length of time that fat has been in the stomach.

With the hope of obtaining some conclusive evidence, the experimental work of Mendel and Baumann on the possibilities of fat absorption through the stomach wall has been repeated on adult dogs with the additional precaution of determining the fat content of the lymph of the thoracic duct at the same time. The experimental data thus collected afford evidence that some of the ingested fat can pass through the stomach wall, even if the amount is ordinarily so small as to be practically negligible.

EXPERIMENTAL. Adult dogs which had received their last meal forty-eight hours before the experiment were anesthetized with A. C. E. mixture. A laparotomy was performed and the pylorus was gently ligated with a strip of gauze. A cannula was inserted in the thoracic duct; another cannula for collecting blood samples was inserted in a femoral artery.

[1] International Fellow of the Rockefeller Foundation.

After the first lymph and blood samples were taken, an emulsion of 30 grams olive oil and one egg yolk was injected into the stomach at body temperature.[2] The abdominal cut was closed. Usually the whole operation was completed in one hour or slightly longer. Throughout the period of observation the total fatty acids in both lymph and blood samples were estimated at short intervals. Immediately after the animal was killed four pieces of the stomach, two from the pyloric and fundic regions respectively, were selected for the histological investigation with the intention of securing identical portions in each subject. These blocks were fixed in formalin, sectioned with a freezing microtome and stained with Sudan III. Details of the experiments and some typical protocols are given in the appendix. The approximate location of the specimens selected is indicated in the drawing.

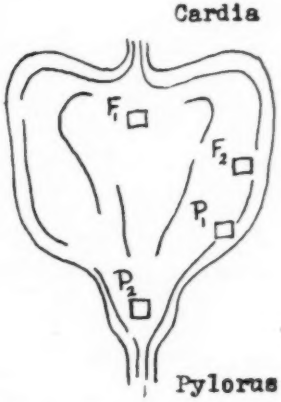

Fig. 1. Sketch of stomach dissected open, showing approximate location of specimens selected for histological examination.

Histological investigation. To avoid the tedious statement of each experiment the results of the histological observations are summarized in the following tables, where the relative abundance of fat droplets in the stomach mucosa is differentiated by the number of plus signs. The minus signs show those cases in which very few or practically no fat globules were observed in the mucosa, while the five plus signs show a case in which the stomach mucosa was so plentifully loaded with fat particles that one could scarcely distinguish the cell-boundaries or the nucleus, even with a favorable counterstain.[3]

From these tables it will be observed that almost invariably after the administration of fat there is some increase of fat droplets in the mucosa after some hours—never in less than five or six hours (cases III, VII, VIII, XIII, XVIII). This result is in contrast with the control cases (table 2), and suggests that in the increase of fat globules in the mucosa after feeding fat evidently depends on the fat introduced into the stomach.

Secondly, in the same animal the portions indicated by F_1, P_2, usually showed more fat particles than those indicated by F_2, P_1. This outcome

[2] As it was found after a few experiments that an increase of fat in the lymph could not be expected within the first two hours the fat emulsion was introduced into the stomach by sound soon after the ligation of the pylorus, in order to avoid unfavorable manipulation to the subject as much as possible.

[3] A photograph of this preparation is reproduced in the appendix. (Fig. 2.)

TABLE 1

Cases in which emulsions of 30 grams olive oil and one egg yolk were introduced in the ligated stomach

DOG	PORTIONS OF STOMACH				THE DURATION FROM FAT FEEDING TO THE END OF THE EXPERIMENT	REMARKS
	F_1	F_2	P_1	P_2		
					hours	
IV	++	+	−	+	$1\frac{1}{12}$	
VI	+	−	+	−	$1\frac{1}{2}$	
V	−	−	−	+	2	
VII	+++++	++++	+++++	+++++	$6\frac{1}{2}$	
III	+++	++	+++	+++	$9\frac{2}{3}$	The epithelial cells contain some small fat droplets
VIII	+++	+++	+++	++++	11	

TABLE 2

Control cases in which various substances were introduced into the ligated stomach

DOG	SUBSTANCES FED	PORTIONS OF STOMACH				THE DURATION FROM FAT FEEDING TO THE END OF THE EXPERIMENT	REMARKS
		F_1	F_2	P_1	P_2		
						hours	
XIV	20 gram lanolin, one egg yolk	+	+	−	+	$5\frac{1}{2}$	
II	30 grams olive oil, one egg yolk	−	+	+	−	6	Injected into the intestine
XII	30 grams mixed* fatty acids	+	+	+	++	$8\frac{1}{2}$	
X	30 grams gum acacia, 10 grams dextrose, 100 cc. water	+	+	−	−	9	
XI	30 grams mineral oil, one egg yolk	+	−	+	−	$9\frac{1}{2}$	
XIII	20 grams lanolin, one egg yolk	+++++	+++	+++	+++++	10	
I	Carmine solution	+	+	−	+	$11\frac{1}{2}$	

* The mixed fatty acids were made from lard.

may be explained by the position of the stomach of the animal during the experiment as well as by the anatomical structure of the stomach; in other words, some portions came into more direct contact with the introduced fat than other regions of the stomach. Regarding the inequalities in the amount of fat in the pyloric and fundic regions in control animals, the condition observed is similar to that described by Baumann, Greene and Skaer. In some animals there seemed to be more fat particles in one region, while in others a reverse condition existed. In the fundic region the gland cells of the upper layer under the superficial epithelium seemed to contain more fat than other parts both in control and fat-feeding cases, while in the pyloric region the gland cells in deep layers were usually the places where plenty of fat particles were observed. It should be noted also, that increase of fat globules in the stomach mucosa was not observed when mineral oil and fatty acids were fed, while there was some increase in the experiment where lanolin was fed.

TABLE 3

Cases in which fat was introduced with the stomach not ligated

DOG	SUBSTANCES FED	PORTIONS OF STOMACH				THE DURATION FROM FAT FEEDING TO THE END OF THE EXPERIMENT	REMARKS
		F_1	F_2	P_1	P_2		
						hours	
XVI	50 grams olive oil	+	−	−	+	6	Given by sound
XV	50 grams olive oil	−	−	+	+	$6\frac{1}{10}$	Injected into the intestine
XVIII	20 grams olive oil	+	++	+++	++++	$11\frac{2}{3}$	Given by sound
XVII	6 egg yolks	++	+	+++	+++	$12\frac{1}{2}$	Given by sound

Chemical investigation. In some of the experiments quantitative determinations of fat in the blood as well as in the thoracic duct lymph were made in order to observe whether there was any change in fat content in these fluids during the experiment. The total fatty acids were estimated by Bloor's latest modified method. The results are summarized in tables 4, 5 and 6.

As will be seen in these tables, the results of the blood analyses were in accord with those obtained by Mendel and Baumann; the blood fat after feeding fat with the stomach ligated showed no change which exceeded the limit of the experimental error. However, the fat content of the lymph collected in the thoracic duct showed a slight percentage increase some hours after fat administration in three cases (III, VII and VIII), i.e., in eight, six and six hours respectively. Although this percentage increase is not remarkable and does not indicate increment in the absolute

amount of fat transported, yet it should not be construed as due to possible errors in the experimental technique. For, as the figures obtained in control dogs show, the percentage fat content in the lymph seemed to be nearly constant no matter what the volume of lymph flow was throughout the entire experiment in the same animal, in spite of the fact that there was some variation in each individual. On the other hand, the

TABLE 4

Content of fat in lymph and blood after introduction of fat (30 grams olive oil + one egg yolk) into the ligated stomach

DOG	LYMPH			BLOOD		REMARKS
	Time	Amount	Fatty acid	Time	Fatty acid	
		cc.	*per cent*		*per cent*	
III	10:30–11:30	19	0.39	11:00	0.44	11:30 fat injected
	11:30– 1:30	3	0.38	1:00	0.40	
	1:30– 3:40	24	0.43	3:00	0.43	12:00 lymph-flow very slow, cannula reinserted
	3:40– 4:40	22.5	0.38	5:00	0.38	
	4:40– 5:40	19.5	0.39	7:00	0.40	
	5:40– 7:40	25.5	0.45	9:00	0.39	
	7:40– 9:40	19.5	0.51			Dog died 9:40 p.m.
IV	9:30			9:40	0.47	Fat given by sound
	10:10–11:10	35	0.40	10:30	0.50	
	11:10–12:10	37	0.39	12:30	0.46	
	12:10– 1:10	20	0.40	1:35	0.48	
VII	9:30			9:00	0.37	Fat given by sound
	11:40–12:40	27	0.35	11:00	0.35	
	12:40– 1.40	29	0.34	1:00	0.39	
	1:40– 2:40	29	0.40	3:00	0.38	Dog died 3:50 p.m.
	2:40– 3:40	26	0.47	3:50	0.40	
	3:40– 3:50	4	0.48			
VIII	9:40			10:00	0.49	Fat given by sound
	10:30–11:30	43	0.40	12:00	0.52	
	11:30–12:30	39	0.41	2:00	0.51	
	12:30– 2.30	42	0.45	4:00	0.53	
	2:30– 4:30	32	0.51	6:00	0.47	
	6:30– 8:30	27	0.63	8:00	0.46	Dog died 10:30 p.m.
	8:30–10:30	29	0.62	10:00	0.50	

histological pictures in these three cases showed plenty of fat globules in the stomach mucosa, while in other cases where no increase of fat in the lymph was observed, (those cases were either control dogs or cases in which the dogs died in the earlier stage of experiment) there also was no noteworthy increase in the fat droplets of the stomach mucosa. Generally speaking, these facts seem to reveal some parallelism between a

slight augmentation of the chyle fat and the histological indications of fat absorption through the gastric mucosa. At least it seems probable that some of the fat injected into the stomach does pass through the stomach wall after a prolonged time in certain conditions and comes out in the lymph of the thoracic duct, though it may be such a small amount

TABLE 5

Content of fat in lymph and blood in control cases with the stomach ligated

DOG	LYMPH			BLOOD		REMARKS
	Time	Amount	Fatty acid	Time	Fatty acid	
		cc.	*per cent*		*per cent*	
I	9:10					Carmine solution injected into stomach
	10:00–12:00	41	0.43	10:30	0.61*	
	12:00– 2:00	33	0.42	12:00	0.59	
	2:00– 4:00	23	0.40	1:30	0.62	
	4:00– 6:00	20	0.39	3:00	0.60	
	6:00– 8:00	19	0.41	4:30	0.61	
				6:00	0.57	Dog killed by bleeding 8:30 p.m.
				7:30	0.62	
				8:30	0.56	
X	9:00					30 grams gum acacia, 10 grams dextrose, 100 cc. water given by sound
	9:45–10:40	74	0.27	10:00	0.36	
	10:40–11:40	71	0.25	12:00	0.42	2:00 p.m. lymph clotted in cannula
	11:40–12:40	69	0.29	2:00	0.40	
	12:40– 1:40	60	0.26	4:00	0.42	
	1:40– 2:00	10	0.26	6:00	0.38	Dog died 6:00 p.m.
XII	9:00					30 grams fatty acids given by sound
	10:05–10:15	13	0.34	10:30	0.48	
	10:15–11:00	33	0.38	11:30	0.51	
	11:00–12:00	31	0.31	1:00	0.50	
	12:00– 1:30	29	0.29	2:00	0.48	
	1:30– 3:30	27	0.32	3:30	0.46	Dog died 5:25 p.m.
				5:00	0.45	

* The relatively high content of blood fat may be due to the state of pregnancy of dog.

as to be almost negligible when compared with that obtained in cases where fat absorption from the intestine is possible (table 6). Whether the gastric phenomenon is a true absorption in the same sense as is intestinal absorption or it represents merely a mechanical injection into the wall of stomach mucosa cannot be definitely determined by these experi-

ments. The negative results after the feeding of mineral oil and fatty acids are not without significance. The inherent difficulty of fat absorption from the stomach mucosa and the necessarily slow transportation of fat through the stomach tissue should be realized.

TABLE 6

Content of fat in lymph and blood after administration of fat into the non-ligated stomach

DOG	LYMPH			BLOOD		REMARKS
	Time	Amount	Fatty acid	Time	Fatty acid	
		cc.	*per cent*		*per cent*	
XV	11:06–11:36	9	0.35	11:35	0.50	11:36, 50 grams olive oil injected into intestine
	11:36– 1:36	30	0.78	2:36	0.64	
	1:36– 3:36	33	1.48	4:36	0.67	
	3:36– 5:36	26	1.40	5:40	0.68	
						Dog died 5:40 p.m.
XVII	8:45					6 egg yolks given by sound
	11:45– 1:45	63	2.95	12:00	0.52	
	1:45– 3:45	47	2.78	2:00	0.59	
	3:45– 5:45	37	1.36	4:00	0.69	
	5:45– 7:45	31	1.68	6:00	0.60	
	7:45– 9:00	15	1.72	8:00	0.60	Dog died 9:15 p.m.
				9:00	0.61	
XVIII	10:05–10:30	10	0.22	10:15	0.47	10:00, 20 grams olive oil injected into intestine
	10:30–12:30	40	0.54	1:30	0.48	
	12:30– 2:30	35	1.05	3:30	0.49	
	2:30– 4:30	42	1.95	5:30	0.52	
	4:30– 6:30	30	1.62	7:30	0.51	
	6:30– 8:30	46	0.84	9:30	0.50	Dog died 9:40 p.m.
	8:30– 9:30	8	0.72			

CONCLUSION

In experiments on dogs it has been demonstrated that when fat is introduced into a stomach ligated at the pylorus, some of it may pass through the stomach wall and appear in the lymph of the thoracic duct after a prolonged time. The amount of fat ordinarily absorbed in this way is so small as to be almost negligible in the consideration of fat transport in the organism.

ACKNOWLEDGMENT. The writer wishes to express his sincere thanks to Professor Lafayette B. Mendel for his helpful advice and criticism throughout this investigation. Grateful acknowledgment is also due to Professor Winternitz, in whose laboratory the histological study of the sections has been made.

APPENDIX

Protocol 1. Control experiment I. Dog, female (pregnant), 11 kgm.

Last meal was forty-eight hours before the operation. At 9:00 a.m. the animal was anesthetized with A. C. E.; at 9:20 a laparotomy was made, the pylorus gently ligated, 20 cc. of carmine solution were injected into the stomach and the abdominal wound sewed up. At 10:00 a.m. the operation was ended after inserting cannulas into the thoracic duct and in a femoral artery. The dog was killed by bleeding at 8:30 p.m. The results of blood and lymph analyses appear in foregoing tables.

Protocol 2. Control experiment X. Dog, female, 14 kgm.

Fasting for forty-eight hours. Operation at 8:40 a.m. under anesthesia of A. C. E. A laparotomy was performed and the pylorus gently ligated, after which the wound was sewed up. At 9:10 the mixed solution consisting of 30 grams gum acacia, 10 grams dextrose and about 100 cc. water was given by sound. At 9:45 the cannula was inserted into the thoracic duct, and the operation was completed by putting another in a femoral artery for blood samples. Both blood and lymph samples were taken at short intervals. The dog was killed by bleeding at 6:00 p.m. The results appear in foregoing tables.

Protocol 3. Fat experiment III. Dog, female, 13 kgm.

Fasting for forty-eight hours. At 9:00 a.m. the animal was anesthetized with A. C. E.; at 9:30 the pylorus was gently ligated. At 10:30 the operation was ended; a cannula was inserted into the thoracic duct as well as in a femoral artery. After the first samples of blood and lymph were taken at 11:00 and 11:30 a.m., the emulsion of fat consisting of 30 grams olive oil, one egg yolk and about 60 cc. water was injected into the stomach and the abdominal wound was sewed up. At 12:05 p.m. the lymph flow became very slow and at 1:00 p.m. nearly stopped running; then the cannula was taken out and reinserted. The experiment continued. Fifty cubic centimeters saline solution were injected subcutaneously at the following times: 11:45 a.m., 1:45, 4:00, 5:40, 6:55 and 8:20 p.m. The dog died at 9:40 p.m. The results of blood and lymph analyses appear in foregoing tables.

Protocol 4. Fat experiment VII. Dog, male, 11 kgm.

Last meal was forty-eight hours before the experiment. At 8:30 a.m. 6 mgm. morphine were injected subcutaneously. The operation was begun at 9:00 under anesthesia of A. C. E. At 9:30 the pylorus was ligated and 30 grams olive oil and one egg yolk were given by sound as emulsion with water. At 11:40 the operation was ended after inserting a cannula into the thoracic duct and in a femoral artery. Fifty cubic centimeters saline were injected subcutaneously at 10:50, 11:50 a.m. and at 12:50, 1:50 p.m. The dog died at 4:00 p.m. The results of blood and lymph analyses appear in foregoing tables.

Histological findings.

F_1	F_2	P_1	P_2
+++++	++++	+++++	+++++

An histological study was also made of several sections from the intestine to be sure that no ingested fat had passed through the ligation into the intestine. A photograph of one preparation from section F_1 of the stomach is reproduced here:

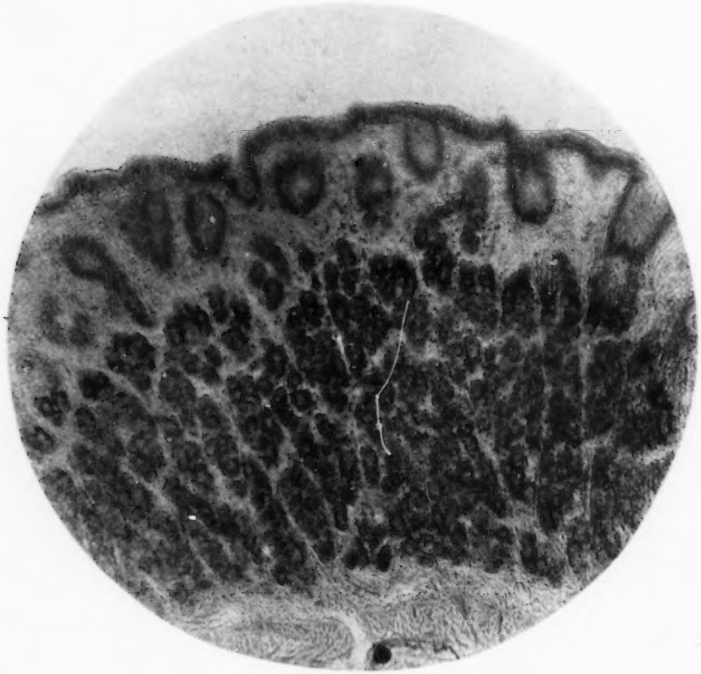

Fig. 2. Photograph of a stained section of section F_1 from the stomach. (See protocol 4, experiment VII.)

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STUDIES IN BLOOD DIASTASES

I. Significance of the Blood Diastase in the Normal Animal

SEYMOUR J. COHEN

From the Department of Pharmacology and Therapeutics, University of Illinois, College of Medicine, Chicago

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Since Magendie (1846) showed the presence of a diastatic enzyme in the blood, it has been the subject of much work and discussion. Considerable importance has been attached to this enzyme by many workers, some (2), (3), (4), (5) claiming it to be a specific hormone with definite function, while others (6), (7) state that it is an end product of metabolism circulating in the blood on its way to elimination by the kidneys.

Since the most probable function of blood diastase is related to sugar metabolism, the present investigation is an attempt to find the function through variations of the blood sugar by the use of drugs.

Method. All the work was done on dogs, which were starved for eighteen to twenty-four hours. The blood was obtained with an aspirating needle by making a puncture directly into the heart. A few crystals of potassium oxalate were added to the blood to prevent clotting. The blood sugar and diastatic activity were performed within ten or fifteen minutes after withdrawal of the blood. The method used was that by Myer and Killian (2) except that the blood sugar was determined by the Folin and Wu (8) method. Duplicates were run on all samples.

Before determining the action of drugs on the blood diastase, the normal diastatic activity of dog's blood was studied. Figure 1 shows first, the diastatic activity of the blood of 45 normal dogs and, second, the relation of the blood sugar to this diastatic activity. In this series of animals, the diastatic activity of the blood varied between 13 and 67, with an average of 37. This is about 12 points higher than human diastatic activity, as shown by Myer and Killian (2). There appears to be no direct relation between the blood sugar and blood diastase, although on closer examination of figure 1, there is a tendency of the blood diastase to increase slightly with an increase in the blood sugar. Also, the average of the diastatic activity in dogs with a blood sugar between 0.054 and 0.116 per cent is 32, while the average between 0.116 and 0.176 per cent is 44—showing that animals having a high blood sugar show a slight in-

crease in diastatic activity of the blood, although there are marked variations.

We next studied the distribution of the diastatic enzyme in the circulating blood. The results are as follows:

	SUGAR	DIASTASE
Whole blood	0.103 per cent	43.0
Blood plasma	0.101	42.0
R. B. C.	0.020	0.5

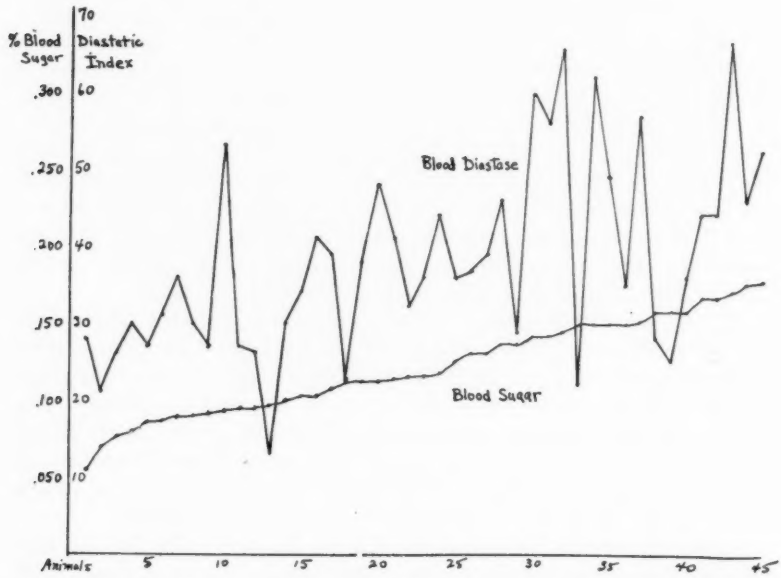

Fig. 1. Showing the relation between blood diastase and blood sugar in 45 animals. In this chart the blood sugar is arranged in consecutive order from the lowest to the highest.

These results indicate that the blood diastase is present practically entirely in the fluid portion of the blood (15). The R. B. C. contain practically no diastase as they contain very little sugar. This enzyme is present only in those organs containing sugar or glycogen.

The effect of hyperglycemia on the blood diastases. There are two methods of producing hyperglycemia: one by breaking down the stored glycogen into glucose, and the other by introducing carbohydrates into the stomach and their absorption into the blood stream. For convenience, we have

called the first method endogenous hyperglycemia, and the second one, exogenous hyperglycemia. The first or endogenous hyperglycemia can be produced by asphyxia, direct stimulation of sensory nerves, and by the action of certain drugs. Exogenous hyperglycemia can only be produced by feeding large amounts of carbohydrate, or injection of glucose intravenously.

Adrenalin is one of the most common drugs used to produce hyperglycemia by breaking down the stored glycogen in the liver, muscles and other organs, to glucose. In a large series of animals, we injected adrenalin subcutaneously as follows:

A normal animal which was starved for eighteen to twenty-four hours was used. The normal blood sugar and blood diastase were determined. Then 2 cc. of 1–1000 adrenalin were injected subcutaneously, and samples of blood were withdrawn at one, two and three hour intervals.

A typical result:

	BLOOD SUGAR	BLOOD DIASTASE
Normal	0.125	46.0
Injection of 2 cc. (1–1000) adrenalin		
1 hour after	0.260	24.0
2 hours after	0.207	28.0
3 hours after	0.187	27.0

The hyperglycemia produced by adrenalin is associated with a corresponding decrease in the blood diastase, which is not, however, absolutely proportional. It appears that as adrenalin produces an increase in the blood sugar, there is a marked decrease in the blood diastases. These results are contrary to those of most investigators (3), (9), (10), (12), but in a large series of animals our observations were practically the same in every case. We are at a loss to account for these negative results of other investigators.

The next question arose. *What becomes of the diastases in the blood during this adrenalin hyperglycemia?* There are possibly three ways to account for this loss. First, they may be destroyed; second, they may be eliminated by the kidney; and third, they may be transferred or absorbed by some other organ in the body.

We have no definite way of determining whether these enzymes are destroyed, for we do not know what end products they might produce. Therefore we must leave this phase of the question open.

During the hyperglycemia, this enzyme might be eliminated in the urine in the same manner as the sugar. In a series of animals we measured the urinary diastase before and after adrenalin, and found that there was practically no change in the urinary diastase, while there was marked

increase in the urinary sugar. From these observations we have concluded that the fall in blood diastase during adrenalin hyperglycemia is not due to elimination of these enzymes by the kidney.

The most probable explanation is that the blood diastases are transferred to the organ or organs where there is an immediate need for their action. It is assumed that the adrenalin hyperglycemia is brought about in the liver by the stimulation of the sympathetic nerve endings by this drug, with the conversion of glycogen to glucose. This nervous action might be assisted or aided by a hormone action, in which the blood diastases are mobilized from the circulating blood to the organs in which this action of conversion of glycogen is taking place. This action is similar to the secretion of gastric juice, in which the flow of juice is initiated by the nerve impulses carried down the vagus, and then aided by the action of gastrin.

As the liver contains the largest percentage of stored glycogen in the body, this organ should show a marked change in the tissue diastase during this hyperglycemia if our theory be correct. With this idea in view, we studied the liver tissue diastase in relation to the changes found in the circulating blood. The method was as follows:

A dog was anesthetized. A sample of blood was obtained and the blood sugar and diastatic activity were determined. At the same time a piece of liver was removed, and the diastatic activity of one gram of this organ was also determined. Then 2 cc. of 1–1000 adrenalin were injected, and after one and one-half hours, the same procedure was repeated.

RESULT	BLOOD SUGAR	BLOOD DIASTASE	LIVER DIASTASE
Before adrenalin	0.187	41.0	33.0
After adrenalin	0.250	35.0	63.0

These results show that adrenalin causes its usual hyperglycemia, with a relative decrease in the blood diastase, and at the same time there is an increase in the tissue diastase of the liver. Now, if we assume that the tissue produces the diastases and pours them into the blood, there is no reason to doubt why the tissues can not re-absorb (10) the diastases again and make use of them. This is analogous to the action of gastrin, which is formed in the gastric mucosa, poured into the blood, and taken up by the gastric glands, where it has its specific function. This change in the liver tissue diastase indicates that the circulating diastases are drawn upon in cases of emergencies, or of increased activity from the organs where they are produced to the organs where there is an immediate need for their action. This, we believe, is the function of the blood

diastases. They are produced by practically all organs containing glycogen, and poured into the blood, where they circulate, some being eliminated by the kidney, while most of them are ready to be mobilized in the organ or organs where there is an active or increased destruction of glycogen into glucose.

Most investigators, especially the clinical men, have, we think, a wrong conception of the function of these blood diastases. The blood diastases are inactive. It is only when they are in the tissue, close to the glycogen, that they become active. Therefore, a larger amount of blood diastase (consequently a small amount of tissue diastase) would indicate a decreased activity of the organs of that animal; a small amount or absence of blood diastase (consequently an increase in tissue diastase) would indicate an increased activity of the organs of the animal.

It is interesting to read the clinical reports of many investigators who believe that diabetes should be associated with a high diastatic activity of the blood, but who (4), (5) have found in severe cases of diabetes, and also in diabetes complicated by syphilis (5), a marked decrease in the blood diastases or an entire absence (11). One investigator (4) stated that a low diastatic activity indicated a fatal termination of the disease. Other investigators, however, have reported a high diastatic index in diabetes (2), (5), (13). This point is now being determined in our laboratory.

We next studied the effect of morphine on the blood diastase. This drug also produces a hyperglycemia of the endogenous type. The results were practically the same as with adrenalin, but not as marked. A typical result is as follows:

	BLOOD SUGAR	BLOOD DIASTASE
Before	0.080	30.6
½ gr. morphine subcutaneously		
1 hour later	0.190	24.4
2 hours later	0.125	21.9

The above experiments indicate that drugs producing endogenous hyperglycemia by breaking down the body glycogen to glucose, produce at the same time a decrease in the blood diastase.

The second type of hyperglycemia, that produced by the ingestion of carbohydrate and its effect on the blood diastase, was determined on a series of dogs as follows: A dog that had been starved for eighteen to twenty-four hours was given 5 grams of glucose per kilo body weight, by stomach tube. A sample of blood was taken before the ingestion and at intervals of one hour for three hours following the ingestion of carbohydrate.

RESULT	BLOOD SUGAR	BLOOD DIASTASE
Before	0.142	56.0
5 grains glucose per kilo		
1 hour	0.213	53.0
2 hours	0.230	57.0
3 hours	0.187	56.0

These results indicate that the feeding of carbohydrate and the production of the exogenous type of hyperglycemia do not affect the blood diastase. This is quite consistent with what we stated earlier. Inasmuch as there is no destruction of glycogen in this type of hyperglycemia, we do not get any change in the blood diastase. These results are also in accord with those of other investigators (4), (6), (7), (13), (14).

The effect of hypoglycemia on the blood diastases. The production of hypoglycemia in the animal has recently been aided by the discovery and introduction of insulin into therapeutics. In a series of animals we injected insulin intravenously, and studied the changes in blood sugar and diastatic activity before injection and two to four and six hours after injection, using the same method as with adrenalin.

RESULTS	BLOOD SUGAR	BLOOD DIASTASE
Before	0.142	60.8
5 unit per kilo		
2 hours	0.085	62.0
4 hours	0.057	51.4
6 hours	0.073	49.8

These results show that as insulin produces a marked drop in the blood sugar, there is also produced a drop in the diastatic activity of the blood. From most recent information insulin causes the glucose to be more easily oxidized, and hence leaves the circulating blood. This calls for more blood sugar, which is produced by breaking down the stored glycogen in the tissues into glucose. This action calls forth more diastase from the blood, hence there is a fall in the blood diastases during this hypoglycemia. In checking this up we studied the liver tissue diastase in relation to the change in the circulating blood, following the injection of insulin, using the same method as for adrenalin.

RESULT	BLOOD SUGAR	BLOOD DIASTASE	LIVER DIASTASE
Before	0.181	54.3	28.5
½ hour later after insulin	0.157	47.1	44.0
3 hours later after insulin	0.096	31.7	70.5

These results indicate that as insulin causes the blood sugar to be more easily oxidized, with the production of a hypoglycemia, glycogen is broken down into glucose to make up for the deficiency in the blood. This action calls for a mobilization of the circulating diastases to the liver, and consequently there is an increase in the liver tissue diastases. These results are similar to those results produced by adrenalin.

CONCLUSIONS

1. Endogenous hyperglycemia, produced by adrenalin or morphine, is associated with a fall in the blood diastase and an increase in the tissue diastases of the liver.
2. Exogenous hyperglycemia, produced by carbohydrate ingestion, is not associated with any change in the blood diastases.
3. Hypoglycemia produced by insulin is associated with a fall in the blood diastases and an increase in the tissue diastase of the liver.
4. The fall in the blood diastase during adrenalin hyperglycemia and insulin hypoglycemia is due to a mobilization of the circulating diastase to the liver.
5. We suggest that the function of the blood diastases is their assistance in the breaking down of glycogen, and their ability or readiness to be mobilized to the organs where there is an immediate need for their action.

I am indebted to Dr. Hugh McGuigan for his suggestions and criticism of this work.

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PARADOXICAL CARDIAC INHIBITION FOLLOWING LESIONS OF THE EFFERENT VAGAL PATHS

JOSEPH BYRNE

From the Department of Biology, Fordham University, New York

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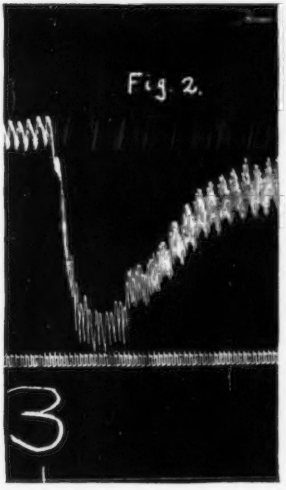
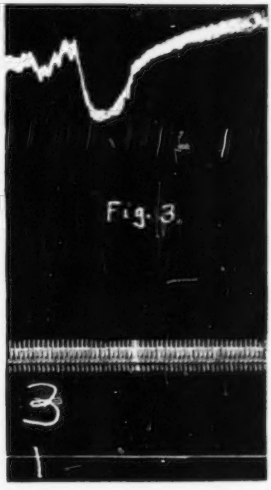
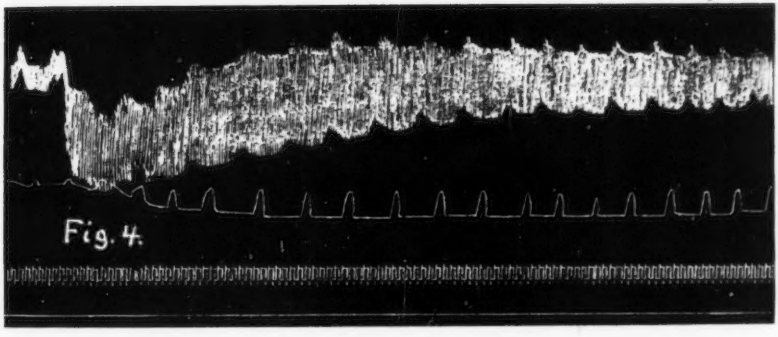
The well-known sensitization to appropriate drugs and agents which the pupil dilator and constrictor mechanisms exhibit respectively after superior cervical ganglionectomy and removal of the ciliary ganglion or the equivalent of these, viz., section of the long or short ciliary nerves, made it apparent to the author that it might be worth while to study the effects upon the heart of section or injury of the efferent vagal paths. The results seem to show conclusively that after section or injury of these paths the intracardiac vagal mechanisms become hypersensitive to pilocarpine, asphyxiation, etc.

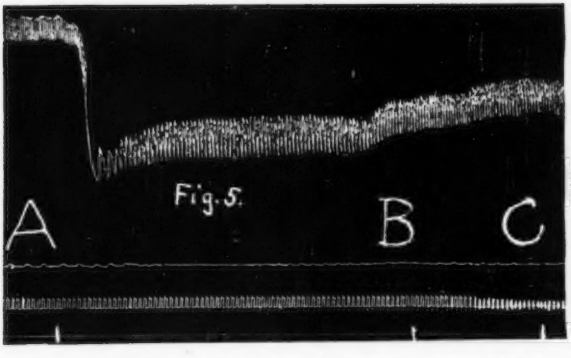
Method. After cauterizing the thyro-arytenoid ligaments, as suggested by Schafer (1), the vagi were sectioned or otherwise injured in the neck and subsequently the effects upon the heart rate, blood pressure, etc., of such agents as pilocarpine, eserine, adrenalin, asphyxiation, extracts of cat's vagal nerves after the manner of Brinkman, van Dam, Jendrassik and Hamburger (2) were recorded and studied. For our purposes pilocarpine hydrochloride given intravenously proved itself to be the most practical and reliable of all the agents. In establishing the presence or absence, in doubtful cases, of vagal hypersensitization only the first or second injection of pilocarpine affords reliable evidence as in many instances after repeated injections of the drug various complicating factors, e.g., sensitization of the vasomotor mechanisms, etc., tend to obscure the study. Compare Brodie and Dixon (3). Cats were used mainly but some experiments were also made on dogs. The results were the same in both although dogs are relatively more susceptible to the effects of pilocarpine than cats. Ether anesthesia. Numerous control experiments were made upon normal cats and dogs. In testing, the average dose of pilocarpine given to animals weighing from 6 to 8 pounds ranged from grain $\frac{1}{200}$ to grain $\frac{1}{100}$ although after ergotoxine grain $\frac{1}{600}$ readily elicited paradoxical inhibition in a cat weighing 8 pounds four days after vagal transection. The blood pressure was taken from different arteries, e.g., carotid, femoral and abdominal aorta.

Experimental facts. Paradoxical inhibition (sensitization) was elicited after the following lesions: a, Simultaneous section of both vago-sym-









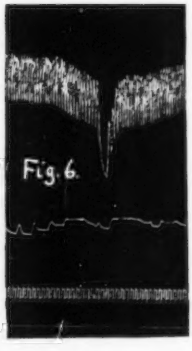


Fig. 1. Dog normal, 15 lbs. Pilocarpine hydrochloride gr. $\frac{1}{100}$ at *2*. Time, seconds in all figures.

Fig. 2. Dog, 15 lbs., 4th day after cutting both vagi. Pilocarpine hydrochloride gr. $\frac{2}{100}$ at *3*. Paradoxical cardiac inhibition.

Fig. 3. Cat, normal, 6 lbs. Pilocarpine hydrochloride gr. $\frac{1}{100}$ at *3*.

Fig. 4. Cat, 8 lbs. 4th day after cutting both vagi. Pilocarpine hydrochloride gr. $\frac{1}{100}$. Paradoxical cardiac inhibition.

Fig. 5. Same cat as in figure 4, after ergotoxine. Pilocarpine hydrochloride gr. $\frac{2}{100}$ at *A*; paradoxical cardiac inhibition; blood pressure 35–50 at *B*; adrenalin mii at *C*.

Fig. 6. Same cat as in figure 4, after ergotoxine. Pilocarpine hydrochloride gr. $\frac{1}{80}$; paradoxical cardiac inhibition. Lower curve in figures 4, 5 and 6 shows respiratory movements registered through balloon in stomach.

pathetics (7 cats, 1 dog) at periods ranging from twenty-one hours to five days. After spinal transection between roots Ci-ii and C vi and vii as well as after removal of both stellate ganglia and after injections of nicotine and of ergotoxine, pilocarpine continued to elicit paradoxical inhibition whereas after atropin even large doses of pilocarpine (gr. $\frac{3}{100}$ to $\frac{8}{100}$) were without apparent effect upon the blood pressure or upon the rate or amplitude of the heart beat. b, Removal of right ganglion nodosum and moderate injury (stretching without crushing) of left vagus (2 animals). Pilocarpine, etc., failed to elicit paradoxical inhibition after the following lesions: *a*, Exposure and very slight injury of both vagi (2 animals). *b*, Section of one vago-sympathetic and very slight injury of the other vago-sympathetic (2 animals). c, Two hours after section of both vago-sympathetics. In 8 supposedly normal cats pilocarpine failed, in the doses used, to elicit paradoxical inhibition. In one cat a fairly well-marked inhibitory response was obtained. Of two supposedly normal dogs pilocarpine failed to elicit inhibition in one whereas the other (weight 12 pounds) exhibited a faint trace of inhibition for a relatively small dose, grain $\frac{1}{100}$, of pilocarpine. In this latter animal the inhibitory phenomena became enormously exaggerated after lesion of the vagal paths. In 2 cats after section of both vagi clinical studies were made. Hypodermic injections of pilocarpine hydrochloride, grain $\frac{1}{100}$, made on the third and fourth days reduced the heart rate by 103 and 120 beats respectively per minute.

Protocol. Cat 4. Young adult, male, 6 lbs. weight.

DAY	TIME	REMARKS
	p.m.	
	9:30	February 2, 1924. Ether. Removed right ganglion nodosum and slightly stretched, without crushing, left vagus.
2nd	6:10	Ether. Artificial respiration; exposed spinal cord for spinal transection.
	6:20	Cannula in abdominal aorta; closed abdomen.
	6:27	Blood pressure 105–110
	6:27	Pilocarpine grain $\frac{1}{100}$; paradoxical inhibition.
	6:35	Transected spinal cord between roots Ci–Cii.
	7:00	Pilocarpine grain $\frac{1}{100}$; paradoxical inhibition.
	7:01	Blood pressure 60–90
	7:13	Nicotine, mxv of a 1 per cent solution.
	7:13	Blood pressure 90–100
	7:20	Pilocarpine grain $\frac{1}{100}$; paradoxical inhibition.
	7:30	Cats' vagus extract (made by extracting after stimulating the nerve); paradoxical inhibition; large heart beats irregular in amplitude.
	7:30	Blood pressure 50–80
	7:40	Atropin sulphate grain $\frac{2}{100}$
	7:42	Blood pressure 75–85
	7:43	Pilocarpine grain $\frac{2}{100}$; paradoxical inhibition absent.

DISCUSSION AND SUMMARY. The question presents itself: Was the paradoxical inhibition the result of lesion of the efferent vagal paths? The answer is in the affirmative since both vagi had been sectioned. Where one vagus is intact the rôle of the injured vagal afferents must be considered. In such cases, however, pilocarpine fails, in the early days after the operation, to elicit paradoxical inhibition partly perhaps because of increased reflex vagal tonus via the injured vagal afferents. Compare the mechanism of pseudo-paradoxical pupil dilatation (4). Here, however, another factor has mechanistic significance, viz., the bilateral distribution of the vagi in the auricular septum. Compare the distribution of the nerves in other viscera derived from the fore gut (5). Such overlapping distribution, more especially in a small highly differentiated region like that containing the pacemaker, would tend to make the heart more or less independent of efferent impulses reaching it over one vagus provided the other vagus were intact. The net result of such a distribution would be prevention of sensitization at the vagal myoneural junctions as much evidence indicates that the sensitization, e.g., in paradoxical ocular phenomena, is fundamentally conditioned by the failure of access, below a certain minimum, of efferent impulses to the myoneural junctions. Another question arises: Is the paradoxical response to pilocarpine the result of asphyxia incidental to bronchial constriction, increased bronchial secretion, etc.? The paradoxical inhibition, however, sets in a few seconds after the injection of pilocarpine before there is time for such factors to register their effects. The fall in blood pressure that follows the injection of pilocarpine is not necessarily a part of the paradoxical phenomenon as it occurs in normal animals and is usually attributed to the slowing of the heart. This explanation is unsatisfactory in view of the fact, well shown in many tracings, that the fall in blood pressure precedes the cardiac slowing and seems to be quite independent of it. When the blood pressure is already low, as after repeated injections of pilocarpine, etc., pilocarpine instead of causing a fall causes a rise in blood pressure which may still be accompanied by marked slowing of the heart beat. In asphyxiation which is a powerful vaso-constrictor excitant, pilocarpine still elicits paradoxical slowing of the heart and at times even a rise in blood pressure. These facts seem to show that pilocarpine has a potential (direct or indirect) vaso-constrictor action which, under ordinary circumstances, is over-shadowed by the circulatory depressant action of the drug. Compare Dale and Laidlaw (6) and Edmunds (7), who have shown that pilocarpine stimulates adrenalin secretion. Under certain conditions, e.g., exhaustion attended by low blood pressure, adrenalin apparently elicited paradoxical cardiac inhibition or its equivalent. Here also is evidence of potential inhibitor action which, under ordinary circumstances, is over-shadowed by accelerator action. Compare Sollman (8), who

adduces evidence to show that under certain circumstances, e.g., calcium deficiency, para-sympathetic drugs may become sympathetic stimulants and vice versa. Compare also the reversal effects on blood pressure and intestinal contractions which Salant and Kleitman (9) found when sodium citrate was administered before or after pilocarpine.

As paradoxical inhibition was readily elicited after spinal cord transection (high and low cervical), after removal of both stellate ganglia, and after nicotine and ergotoxine intravenously, and as it was no longer possible to elicit it after atropin, the conclusion seems warranted that in paradoxical inhibition we are dealing with a phenomenon closely allied in nature and mechanism to the paradoxical ocular phenomena observed after lesion of the efferent pupil dilator and constrictor paths.

CONCLUSIONS

1. Lesions of the efferent vagal paths in the neck induce paradoxical cardiac inhibition or hypersensitization of the intra-cardiac vagal mechanisms to parasympathetic agents such as pilocarpine, etc.

2. Paradoxical cardiac inhibition closely resembles in nature and mechanism the paradoxical ocular phenomena observed after section or injury of the efferent pupil dilator and constrictor paths.

3. The fall in blood pressure that accompanies pilocarpine injections seems to be independent of the slowing of the heart rate as such.

4. Evidence is adduced of the twofold reversible action of sympathetic and parasympathetic drugs.

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THE EFFECT OF INSULIN ON COLD-BLOODED VERTEBRATES KEPT AT DIFFERENT TEMPERATURES

J. M. D. OLMSTED

From the Department of Physiology, University of Toronto

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The type of convulsion in mammals which follows an overdose of insulin is now well known. Similar convulsions have been reported by Krogh (1) to occur in frogs, and the chief events of such convulsions have been outlined by Huxley and Fulton (2). I had also been working on this same problem, and this paper gives the results I obtained on both fish and frogs.

A vigorous reaction appears in the common catfish, Ameiurus nebulosus, about two days after injection of insulin when kept at room temperature. Doses calculated on the basis of rabbit units, i.e., 1 unit per kilogram body weight, appeared to have no effect. It was also evident that a dose which was just ineffective at a lower temperature would cause convulsions at higher temperatures. For experimental purposes, therefore, a uniform dose of 0.2 cc. of 20 unit insulin, which in every case did produce convulsions, was used. The fish were all about six inches in length, taken from a fresh water pond near Woods Hole, Mass., and this part of the work was done at the Marine Biological Station.

The first noticeable effect of insulin on the catfish was the darkening of the skin. Catfish which have been kept for a few days in the light always lose their dark color and change to a light tan, becoming almost transparent. The melanophores of the fish at the time of injection of insulin were in this contracted condition, but on the following day the melanophores had expanded fully and the fish became jet black and remained so for four or five days. A few hours before the actual convulsion the fish appeared weak and unable to swim against a gentle current of water. At this time also it was practically insensitive to stimuli, such as touch, etc. While thus being passively carried around the tank, suddenly it would dart through the water at a very rapid pace, often gaining such momentum that it was carried out over the edge of the tank. Then occurred a period of inability to maintain its equilibrium. If it was quiet at this time it would roll over very slowly to one side, righting itself with a quick jerk, or the tail would rise slowly until the fish stood on its head, then suddenly the tail would be brought down with a jerk or the

fish would start swimming. The sudden dashes through the water soon took a spiral course, and the fish progressed in corkscrew fashion. The rotation noted in some twenty fish was in general to the animal's right. A few individuals were seen turning to the left and one turned over straight backwards. When the turning movements ceased the fish either floated motionless head out of water and tail straight down, or sank to the bottom and lay motionless on its side. The gills ceased to move and the animal was quite inert. After several minutes the eyes began to roll, then the gills to move, the fish now recovered its balance and usually swam along the surface, mouth out of water gulping air vigorously. Frequently instead of dashing about the vessel in a spiral course the fish would give a convulsive shudder and move backwards in a series of jerky movements. Immediately after a convulsion the fish became weak again and also refused to respond to stimuli such as touch, vibration, etc. A few hours later, however, it became hypersensitive and dashed about the tank even when the table was struck at some distance from it. The effect of the insulin was found to wear off gradually if the fish was left at room temperature.

To determine the relation of temperature to the time for convulsions the fish were kept for a few hours in water at the desired degree and then injected with insulin. Controls were used in each case either normal, or injected with an equal volume of water. The control animals exhibited no peculiar symptoms after becoming ajusted to the temperature except that they were unable to survive the highest temperature, 29° to 30°, for more than six hours. It has been shown by Britton (3) that salt-water fish withstand an exposure to a temperature of 25°C. with little difficulty, but that at 30° death follows unless the temperature is reduced. This makes it doubtful whether the effects at the highest temperature were due to insulin or to the heat, but in all cases, however, the injected fish gave signs of distress and feeble convulsions before it became evident that the heat was affecting the control fish.

The curve in figure 1 shows the effect of temperature on the time of the onset of convulsions. The points are the average of 4 or more readings. At 21° the time varied from fifty-one to fifty-seven hours, and at the highest temperature two and one-half to five hours.

All the insulin fish which had been kept at room temperature and those which had been at 25° but were returned to water at room temperature after having convulsions were found to recover perfectly, and as a rule showed no further convulsions after twenty-four hours. Nevertheless they remained black for several days. If, however, they were allowed to remain at the higher temperature or were again placed in water above 25°C. they succumbed to the heat in a very few hours.

The majority of the fish which had been at 29°C. continued to show convulsions when returned to water at room temperature and all died within a very few days. Injection of saturated glucose solution caused temporary relief only. Controls returned to water at room temperature from 29° continued to live indefinitely with no evident ill effects.

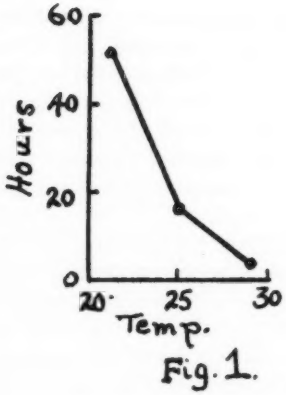

Fig. 1.

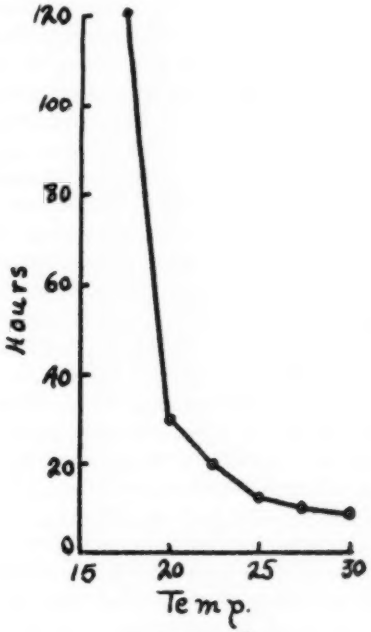

Fig. 2.

Fig. 1. The ordinates represent hours after injection of insulin at which convulsions appear in catfish; the abscissae represent temperatures at which the fish were kept.

Fig. 2. The ordinates represent hours after injection of insulin at which convulsions appear in frogs; the abscissae represent temperatures at which the frogs were kept.

Gold fish were also tried but did not prove to be satisfactory material. A dose of 0.2 cc. insulin injected into gold fish at 18.5° caused no change in their behavior. Nineteen days later a second dose was given and the temperature raised to 21°C. After fifty hours one showed loss of equilibrium, swam in narrow circles with mouth out of water, and occasionally gave a convulsive shudder followed by a short dash through the water.

There was never any corkscrew motion. The effect was observed in only one fish and it died five days after the second injection. Other goldfish subjected to the same treatment lived on and behaved normally for over a month.

Turtles also proved unsatisfactory material. These turtles had been without food for two months. Injection of 1 cc. 20 unit insulin had no effect at 21°. Ten days later 2 cc. and 3 cc. were injected. One injected with 3 cc. died five days later, but exhibited no peculiar behavior before its death. All others lived on for over a month.

Krogh found that frogs at room temperature develop convulsions in four to five days. I have noticed in addition to the details given by Huxley and Fulton the following phenomena. Immediately before the convulsion the skin of the frogs becomes lighter in color, the males usually croak, the limbs become rigidly extended as in strychnine poisoning, the eyes are covered by the nictitating membrane, and the pupils are dilated. Suddenly the frog leaps about most vigorously, falling backward and often rolling over or turning back somersaults. This vigorous convulsion lasts only a few seconds. The frog now becomes limp, respiratory and other muscular activity ceases. Often the lungs remain fully inflated so that the sides of the frog are puffed out and the animal floats, nostrils under water and legs hanging limp. Within a few minutes it lifts its nostrils out of water, opens its eyes, flexes its legs and begins to breathe very rapidly. Usually a second convulsion does not take place for an hour or so, when the entire performance is repeated.

I found also that injected frogs left at the higher temperatures all died fairly soon, i.e., within a day of the first appearance of convulsions, while their lives could be saved by returning them to water at room temperature. Also if, after all signs of convulsions had been absent from these injected frogs returned to water at room temperature, they were again transferred to the higher temperature, convulsions again developed within a very few hours. During the week following active convulsions it was noticed that many frogs did not make complete compensatory movements when rotated in different planes. This was especially noticeable in the lack of response of the head to rotation in a horizontal plane. Furthermore the muscles on one side of the body appeared to be somewhat atonic, so that one shoulder was higher than the other, and one hind leg less flexed than its fellow.

The effect of temperature on the time of onset of convulsions is shown in figure 2. The points on the curve are the averages of 5 to 15 readings. The variation from the average was considerable at the lower temperature, $\pm$ 6 hours, while at the higher temperatures the variation was $\pm$ 2 hours from the average. The curve appears to be a right angled hyperbola, and as Huxley and Fulton point out, the indication is that the action of insulin

itself is not altered by the change in temperature. It appears rather, as suggested to me by Dr. Selig Hecht, that some substance is produced, a definite amount of which is necessary to cause convulsions, and that the production of this substance through the agency of insulin is practically a linear function of the temperature.

Huxley and Fulton make no mention of any attempt to correlate insulin convulsions with fall in blood sugar. In mammals a decrease in the blood sugar to approximately 0.045 mgm. glucose per 100 cc. blood is associated with the appearance of convulsions. The blood sugar of frogs at room temperature is so low that it is hardly titratable by the Shaffer-Hartmann or the Hagedorn-Jensen method. The difficulty is not for lack of blood, for in five experiments I used the giant southern bull frog, the smallest weighing 360 grams, the largest 480 grams, and from each of these amphibians 3 to 4 cc. of blood could be obtained from the heart without difficulty. The blood sugars ran in the normals about 0.012 mgm., and after insulin 0.015 to 0.025. One frog was inadvertently allowed to remain in the bath until his rectal temperature was 34.5°C. This frog passed into a condition in which its muscles were so contracted as to resemble heat rigor. It was revived with application of cold water so that it became limp. It began to breathe, and readily gave the wink reflex. The blood sugar of this frog proved to be 0.117 mgm. Consequently the percentage blood sugar was calculated in several frogs in water at 28 to 30°C. for varying periods. The large bull frogs developed a rectal temperature of 29.5°C. within one and one-half hours but their blood sugar was still 0.012 mgm. Seven smaller frogs kept for two days at 28°C. gave values of blood sugar ranging from 0.032 to 0.112, with an average of 0.056. Nine similar frogs also kept for two days at 28°C. then injected with 0.1 cc. 10 units insulin gave at the time of convulsions blood sugars ranging from 0.032 to 0.091, with an average of 0.048. It is evident that in frogs insulin has no pronounced effect on blood sugar which has been increased above the normal by keeping the animals at an abnormally high temperature.

SUMMARY

Insulin convulsions in fish and frogs are described. The curves obtained by plotting the temperature against the time elapsing between insulin injection and the appearance of convulsions confirms the conclusion of Huxley and Fulton that the "activity of insulin itself is not essentially altered by temperature, but that its speed of action is dependent upon the the metabolic rate of the animal itself." Insulin does not cause a lowering of the abnormally high blood sugar of frogs kept at high temperatures.

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THE EFFECT OF INSULIN ON THE BLOOD

I. Changes in Oxygen Saturation, Percentage Hemoglobin and Oxygen Capacity

J. M. D. OLMSTED and A. C. TAYLOR

From the Department of Physiology, University of Toronto

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During the experiments reported by Olmsted and Logan (1) the observation was made that after an overdose of insulin decerebrate cats die of respiratory failure, and their arterial blood becomes dark and venous in character. Similar observations were made on decapitate cats in which the blood sugar had been lowered below convulsion level, and it was found that the samples of blood taken in the neighborhood of the convulsive level appeared under-aerated, were dark in color and clotted easily. The suggestion was made that anoxemia might be an important factor in the production of the convulsion, and that the symptoms might possibly be due to the fact that "through the lowering of the blood sugar certain oxidative processes become depressed to such a degree that the brain cells, which are known to be especially susceptible to lack of oxygen, are affected in much the same manner as in asphyxia." Rabbits which have marked insulin hypoglycemia with convulsions show similar darkening of arterial blood.

These were simply visual observations. With the aim of obtaining quantitative data on this subject the work outlined in this paper was undertaken. The experiments may be reviewed under three heads: 1, decapitate cats: 2, decerebrate cats; and 3, rabbits.

1. Decapitate cats. The decapitations were performed in the usual way, the head being entirely removed, in most cases without any appreciable loss of blood. Artificial ventilation had, of course, to be supplied. The aeration was kept constant as far as possible, and judging from normal and decerebrate cats, 72 liters of air per hour were found adequate. To be on the safe side, aeration was kept on an average at 96 liters per hour and never allowed to fall below 72 liters per hour. Furthermore it was found that the effect of changes in temperature on the percentage hemoglobin was so pronounced that care had to be taken to keep the temperature from varying through more than 1°C. We found almost invariably that the temperature was high immediately after decapitation, probably due

to struggling during the administration of the anesthetic. The preparations lasted eight to thirty-six hours. Throughout our experiments a uniform dose of 1 cc. of 10 unit insulin was given, regardless of the size of the animal. The average weight of the cats used was 3 kilograms so that this was always a considerable overdose. Blood was taken at intervals of half an hour or more and in order to obviate any error due to excessive exsanguination not more than 0.5 cc. was taken at one time. Estimations of percentage saturation with oxygen, oxygen capacity, percentage hemoglobin and blood sugar were made by micro-methods. For determining the percentage saturation and oxygen capacity we employed Barcroft's (2) differential blood-gas apparatus for 0.1 cc. We found that we obtained more satisfactory results in doubling the quantity of blood recommended. The actual amount used was 0.19 cc. The blood was collected directly from the carotid artery into a capillary pipette. The first and last portions of each sample were drawn off into a crucible containing a few grains of potassium oxalate. This fraction was used for the hemoglobin and blood sugar determinations. The middle portion of the blood in the capillary pipette, which had not been exposed to air was delivered under the solution of ammonia in the Barcroft bottle. The technique was that outlined by Barcroft, the shaking being continued until three consecutive readings remained the same. The hemoglobin was estimated by the carbon-monoxide method of Palmer (3), the colors being compared in a Dubosecq-Leitz colorimeter. Blood sugars were estimated by the Hagedorn-Jensen method (4).

The numbers in table 1 give the percentage saturation in eight control preparations, and table 2 in ten preparations injected with insulin. Comparison of these tables shows that although the readings are not widely divergent, there is a distinct tendency in the injected preparations for the percentage saturation to become lower about three hours after administration of insulin. This is shown for three individual cases in figures 1, 2 and 3 where the percentage saturation is represented by lines composed of dots and dashes. This tendency is most suggestive when one remembers that the animal was being uniformly aerated and to a degree more than sufficient to keep the alveolar oxygen tension at a maximum.

Quite conclusive results were obtained in the experiments dealing with the percentage hemoglobin and oxygen capacity. In figures 1, 2 and 3, the percentage hemoglobin is plotted in heavy lines. Figure 4 is a composite graph of the observations on nine preparations, three controls plotted in broken lines, and six injected with insulin plotted in solid lines. The hemoglobin of the first blood taken is reckoned as 100 per cent, and all subsequent samples are compared with this first one as the standard. This blood was usually taken one and one-half to three hours after decapitation.

Figure 1 shows a typical preparation which was observed for eleven hours. Insulin was then administered and the observations continued for a further period of eleven hours. The percentage hemoglobin fell sharply at first, then gradually rose until at the time of injection of insulin it was again 100 per cent. Two and a half hours after insulin it had risen to 130 per cent, and remained at a high level throughout the rest of the experiment.

TABLE 1

Percentage oxygen saturation of hemoglobin in control decapitate cats

CAT NUMBER	HOURS AFTER DECAPITATION																	
	½	1½	2	2½	3	3½	4	4½	5	5½	6	6½	7	7½	8	8½	9	9½
14				95	92						93		92					
21				95		89		87		82				89				92
23				93				91		89			95			92		90
25			95			94		93		93		93	93					
52	91						93				93							
55		87	90		94		89		96		96							
56	98	98		98	98		95		95		95		95		95		95	96
66				82		85		90		94			92		91			

TABLE 2

Percentage oxygen saturation of hemoglobin in decapitated cats injected with insulin

CAT NUMBER	HOURS BEFORE INSULIN				HOURS AFTER INSULIN												
	2	1½	1	½	½	1	1½	2	2½	3	3½	4	4½	5	5½	6	6½	7
15				95				93		87		89						
16				89			91	88.5		84								
18					95	88	89	90		89			88					
25	93		93	93			94	93		91								
58			98	97		97		92	98		94		95		90		96	
58a	95		92	93			91	91		95		90				97		
59			97	95		96	90		91		96		92		92		93	
60				98				95			92		93			95		95
61			98	95		89		96		87		85		98		91		
66a				95	87					85			87					

A similar rise after insulin administration is shown in figure 2. Two and one-half hours after insulin the per cent hemoglobin increased from 105.5 per cent to 128.3 per cent within thirty minutes' time. It continued to rise until it reached a maximum at 144.3 per cent five and one-half hours after insulin; at this time sugar solution was injected subcutaneously. The per cent hemoglobin remained, however, at a high level (136 per cent) for fifteen hours, when a second dose of insulin was given. This again produced a rise in hemoglobin from 137.5 per cent to a maximum of 150.3 per cent three hours later.

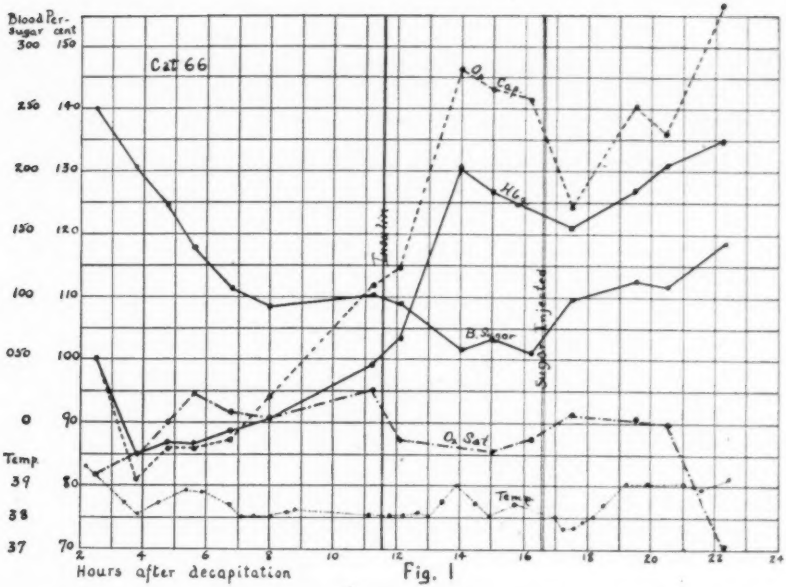

Fig. 1. Cat 66. Decapitate control for 11 hours. Blood sugar falls slowly to normal; per cent hemoglobin and O_2 capacity fall at first, then return to normal; O_2 saturation rises and remains fairly level; after insulin, blood sugar and O_2 saturation fall, hemoglobin and O_2 capacity rise sharply; injection of sugar causes the reverse effect, but only temporarily. Temperature kept about 38°C.

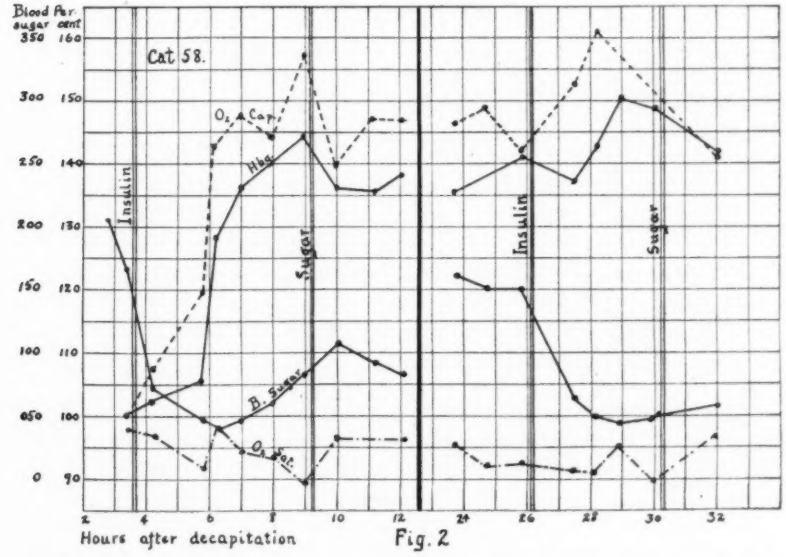

Fig. 2. Cat. 58. Decapitate with insulin. Same effects as in second half of figure 1.

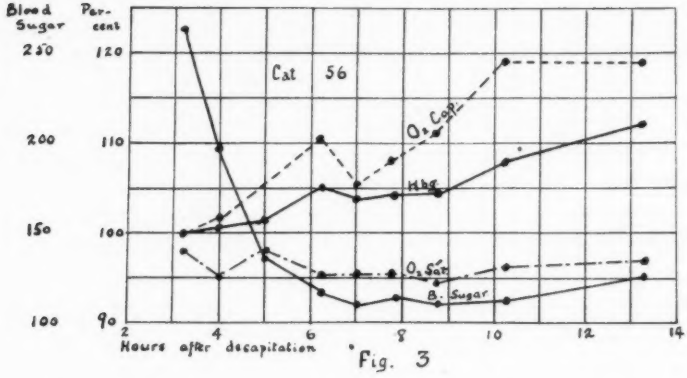

Fig. 3. Cat 56. Decapitate control. Same effects as in first half of figure 1

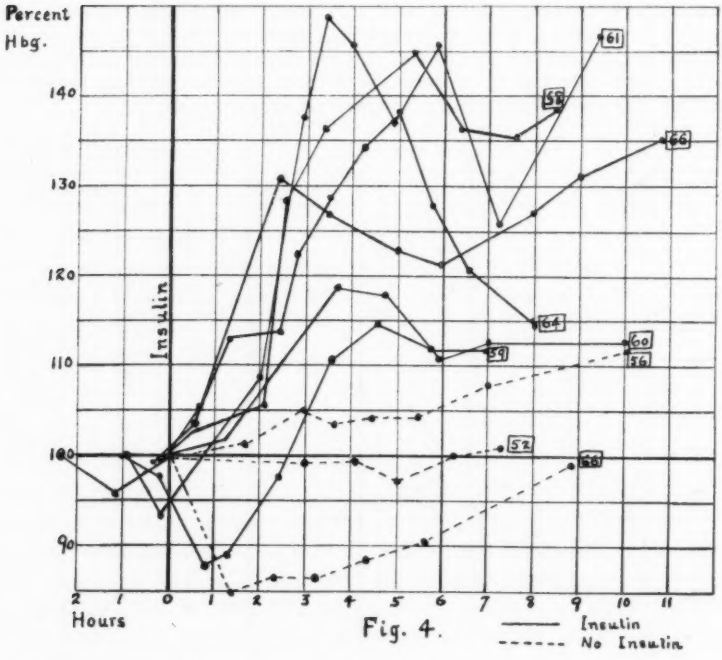

Fig. 4. Composite chart showing effect of insulin in causing abrupt rise in hemoglobin in 6 preparations; 3 controls show no such rise.

In the control preparation, figure 3, the percentage hemoglobin rose gradually as it is seen to do in figure 1 before the administration of insulin. The highest point which it reached was 112 per cent and three hours later (not represented in graph) it was the same.

The composite graph in figure 4 perhaps brings out more clearly the contrast between control and injected preparations. In none of the three controls does the hemoglobin concentration show the sharp rise which is characteristic of all the injected preparations, nor does it reach as high a level.

Injection of a saturated solution of dextrose sometimes caused no change in the hemoglobin concentration, but more often a temporary slight fall. Later, however, there was usually a further rise in hemoglobin concentration. This is interesting in view of Keith's recent paper (5) on dehydration of the body following intravenous injection of glucose when the per cent hemoglobin rose as high as 140 per cent of the normal.

One feature of these hemoglobin curves is the frequent fall during the first three hours after decapitation. This appears to be correlated with the abnormally high rectal temperature and high blood sugar of the preparation during this time as shown in figures 1 and 3.

The percentage oxygen capacity follows the percentage hemoglobin but with more variation due to the fact that very small differences in the absolute amount of oxygen given off with ferricyanide from so small a quantity of blood, make very great differences when calculated as per cent. The parallelism between the two is seen in figures 1, 2 and 3. The sudden rise in oxygen capacity after insulin was observed in all our preparations without exception, and followed the hemoglobin concentration faithfully. In every case the percentage oxygen capacity reached a higher maximum than the percentage hemoglobin. We are not sure that this is significant because of the difficulty in obtaining accurate determinations of the O_2 capacity with such small amounts of blood, and further, the first sample of blood taken may not be absolutely normal.

In many of these decapitate preparations, especially in those which were hypersensitive due to over-aeration, indications of convulsive spasms were noted three to five hours after insulin. One preparation (cat 58, fig. 2) became irritable and showed frequent scratch reflexes four and one-fourth hours after insulin. Twenty minutes later it showed great hypersensitivity, vigorous scratching movements and sudden violent spasms in which both hind legs were drawn up alongside the body; the forelegs were either rigidly extended or were engaged in a peculiar, rapid shaking motion; the muscles of the shoulder and neck were rigid; the tail curved forward under the body; the penis was erect; the abdominal muscles were in marked spasm. Between convulsions the cat was quiet but the slightest touch, and even blowing on its side, would precipitate another spasm.

This lasted throughout one hour when concentrated sugar solution was injected subcutaneously. Following this there were no further convulsions and fifteen minutes later the animal was no longer hypersensitive. In other animals the characteristic features were marked extension of both hind and fore limbs, urination, defecation and muscular twitching. In still others there was no effect whatever, not even hypersensitivity. It has been stated that the chief effect of insulin hypoglycemia is on the medullary centers (1), but from these observations it would appear that after insulin the cord also may become hyperexcitable.

These results on decapitate animals seem to show that there is a tendency for the blood to become slightly less saturated about three hours after insulin, although the ventilation is clearly adequate; on the contrary, the concentration of hemoglobin and the oxygen capacity increase markedly. Furthermore, about this time or just following, hypersensitivity and convulsive spasms often develop, and the blood sugar is at its lowest.

2. Decerebrate cats. These preparations were made by the usual Sherrington guillotine method, the cut being at the level of the anterior corpora quadrigemina. After the anterior part of the skull had been taken away the pituitary body was removed from the pituitary fossa with curved forceps. In private correspondence Dr. W. B. Cannon has pointed out that this procedure in all probability injures the hypothalamus and that the difference in effect on the blood sugar of high and low decerebrations is not due to the removal of the pituitary as Olmsted and Logan had thought but to injury to this region of the brain (see Bailey (6)). In decerebrate preparations the medulla is intact and the animal breathes through its own efforts. Therefore the percentage oxygen saturation of the hemoglobin is much more significant than in decapitate preparations where a constant volume of air is artificially driven into the thorax. The interesting feature is that just before convulsions occur the ventilation increases enormously, yet the percentage oxygen saturation of hemoglobin is actually lowered. This is brought out especially well in figure 5, which is the best record out of nine preparations. We have found in all the decerebrate cats which had insulin convulsions that the oxygen saturation became definitely lowered just about the time the convulsions began. Table 3 gives the data on this point. It will be noted in cat 3 that insulin was given, no convulsions followed, and the percentage oxygen saturation remained high. A further dose of insulin caused convulsions and a fall in percentage saturation was noted. In cat 47 two doses of insulin failed to cause convulsions and there was no fall in per cent saturation. It was found, however, that in only one case, cat 38, did the oxygen saturation fall low enough to be able to bring about convulsions due to asphyxia. From these results it seems certain that the assumption that convulsions are due primarily to anoxemia and only secondarily to injection of insulin,

is incorrect. The evidence points to the conclusion that the appearance of convulsions and the anoxemia are both consequences of insulin injection.

In only three preparations was the hemoglobin and oxygen capacity estimated. One of these is inconclusive. The animal was perfectly quiet until 6 hours after the injection of insulin when it gave signs of respiratory failure accompanied by slight movements of the legs. The hemoglobin and oxygen capacity varied considerably through the experiment, a maximum rise to 110 per cent occurring five hours after insulin. This was followed by a marked fall. In the other two cases, one of which is shown in figure 6, the results were similar to those obtained in decapitate cats.

3. Rabbits. Inspection of arterial blood taken from the carotid artery of rabbits at the time of insulin convulsions showed that, as had been

TABLE 3

Decerebrate cats

CAT NUMBER	HOURS BEFORE INSULIN				HOURS AFTER INSULIN													
	2	1½	1	½	½	1	1½	2	2½	3	3½	4	4½	5	5½	6	6½	7	7½	8
3				89								92	*						83†	
4								96			87†									
32		91		90		82	81	69	88		85†									
33			86			88	86	86	88	88	80	75†		81				89		
38				91	92			83†	38											
45				88		91	92	96	81											
46						96		94	89†	91	89									
47				98	96		93		94		95*		98		94			*	95	93
62				98		95		91		96	94		94	97			93			

*Another injection of insulin.
†Convulsion.

observed in cats (1), the color was often much darker than normal arterial blood, and in several cases it took on a brighter hue when shaken in air. This observation has been repeated on a number of occasions in the laboratory of the Insulin Committee of the University of Toronto. If a rabbit shows convulsions within one and one-half hours after injection of insulin, there is seldom difficulty in obtaining blood from the ear, but in many cases if convulsions continue, or the rabbit is not in good condition, at three hours after insulin it is almost impossible to get blood from the ear, and when obtainable it is very dark even for venous blood, and clots very rapidly. This does not always appear to be the case, however, and frequently no change whatever is noted.

Experiments performed on 5 rabbits after insulin and 4 controls, in which the per cent saturation was determined by the Haldane blood-gas apparatus with 2 cc. of blood (7), gave no significant results. The controls

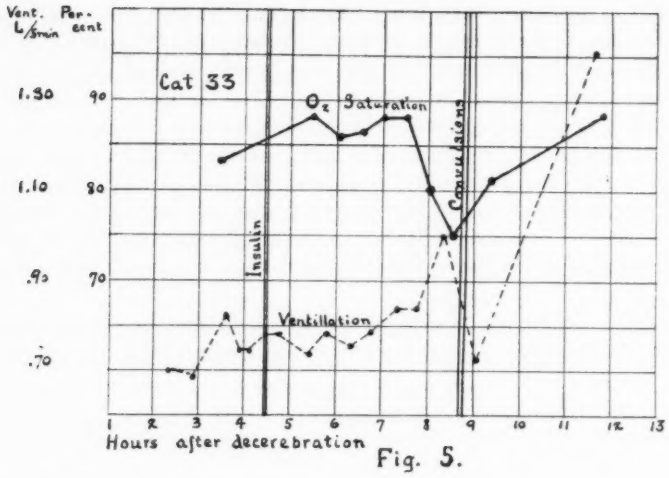

Fig. 5. Decerebrate cat 33. Ventilation increases just before insulin convulsion, but O_2 saturation falls.

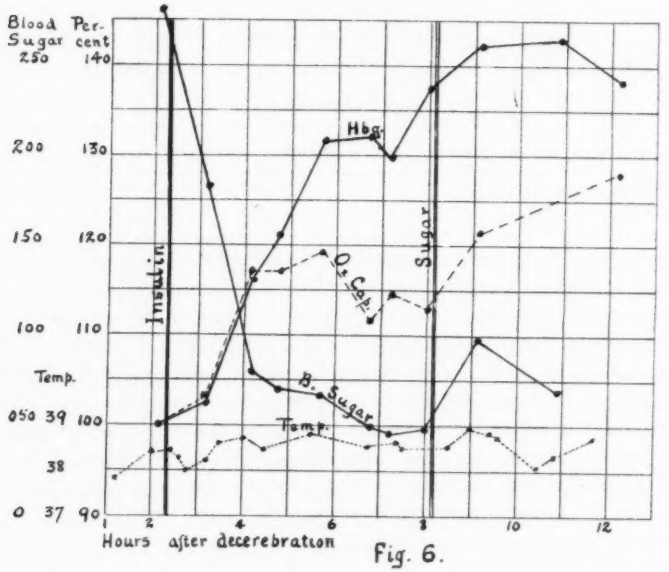

Fig. 6. Decerebrate cat 67. After insulin, hemoglobin and O_2 capacity rise markedly; blood sugar falls.

were found to vary through as wide a range as the animals injected with insulin.

We examined the blood of 7 rabbits used for assay work for changes in hemoglobin concentration and found practically no change one and one-half, three and five hours after insulin, using the sample taken before insulin as a standard for comparison. None of these rabbits developed convulsions since the dose was too light, and the blood sugar of only three of them fell below 0.040. This occurred in the one and one-half and three hour samples only. The five hour sample showed 0.085, 0.076 and 0.057 per cent blood sugar. Nevertheless the failure of these rabbits to show the increase in per cent hemoglobin and oxygen capacity that we had found in the cats was not due to the small dosage of insulin for it was found that rabbits which developed violent convulsions following injection of 1 cc. of 10 unit insulin also failed to show this effect. Three such rabbits were starved, another was well fed. In table 4 will be found the records of these four. Only the starved rabbits developed convulsions, and there was no increase in hemoglobin concentration or oxygen capacity, although exactly five hours after insulin it was difficult to get blood from the ear, and recourse had to be taken to cutting the skin of the throat and bleeding from the jugular vein. This sample of blood, however, did not appear extraordinarily dark, nor did it clot more easily than usual. Out of 10 starved rabbits with varying doses of insulin, 9 showed a slight fall in concentration of hemoglobin and a similar fall in O_2 capacity (table 4). There is very great variation in rabbits. Some will not develop convulsions at all, even with ten times the normal dose of insulin; others develop convulsions within half an hour after the injection of a very small amount.

Our findings on rabbits show that there is no significant change in oxygen saturation; that the concentration of hemoglobin and the oxygen capacity either remain unchanged or fall slightly.

Discussion. We have found that decerebrate cats show some decrease in O_2 saturation of the arterial blood after insulin at the time when blood sugar is low. This decrease is, in the majority of cases, too slight to be responsible for convulsions. This is borne out by the fact that we have been unable to demonstrate any such decrease in O_2 saturation in rabbits. It is strange that the apparent venous character of the arterial blood observed in many rabbits at the time of insulin convulsions has not been confirmed in quantitative experiments. The results on decapitate cats are inconclusive though there appears to be to some extent the same tendency in them as in decerebrate cats.

Macleod (8) has pointed out two ways in which insulin might effect the oxidative processes in the medullary centers in order to produce convulsions. "The lack of oxygen in the nerve cells may result either from a failure in the supply carried to them by the blood or to the development

TABLE 4

CONDITION OF RABBIT	HOURS AFTER INSULIN														
	1½ hours			2 hours			3 hours			4 hours			5 hours		
	Blood sugar	Per cent hemoglobin	O_2 capacity	Blood sugar	Per cent hemoglobin	O_2 capacity	Blood sugar	Per cent hemoglobin	O_2 capacity	Blood sugar	Per cent hemoglobin	O_2 capacity	Blood sugar	Per cent hemoglobin	O_2 capacity
1. Starved 1 day, light dose insulin	0.057	99.9	—	—			0.52	98.8					0.71	98.5	
2. Same as 1	0.033	98.7	—	—			0.28	100.7					0.76	93.1	
3. Starved 36 hours, 1 cc. insulin	0.110	102.7	98.8	0.061	96.6	97.7*	0.047	97.5	97.7	0.050	98.3	91.1	0.057	100.7	95.4
4. Fed, 1 cc. insulin	0.082	98.5	102.6	0.079	98.7	105.3	0.084	97.5	108.2	0.091	98.1	104.8	0.130	100.4	104.3
5. Starved 3 days, 1 cc. insulin		95.3	100.0	+	95.3	87.3					92.3	81.7			
6. Same as 5		98.0	80.0	+				97.8	96.0		86.7	75.9			

*Convulsions.

within their protoplasm of some condition which renders them incapable of utilizing the oxygen with which they are supplied." Our experiments show quite definitely that it is not the breakdown in the transport of oxygen by the hemoglobin which is responsible for hypersensitivity or insulin convulsions.

The very definite results, however, on hemoglobin percentage and O_2 capacity of the blood in decapitate and decerebrate cats show that the blood evidently becomes more concentrated just at the time when the blood sugar reaches its lowest level. These observations form a striking parallel with the results obtained by Keith (5) from dehydration experiments on dogs after intravenous injection of sugar. He found that "The venous blood was more difficult to obtain, darker in color and appeared to clot more rapidly than normal. The hemoglobin rose 13 to 40 per cent." There was a marked increase in the viscosity of the blood, but the percentage saturation of oxygen in the arterial blood did not change significantly. He found that occasionally the dog exhibited shivering, general restlessness, hyperpnea, muscular twitchings and passage of stools. All these phenomena were observed in our experiments on decapitate and decerebrate cats injected with insulin. It is possible that the same mechanism is responsible for Keith's results as for ours, since, Banting and Gairns (9) claim that "when the percentage of sugar in the blood is suddenly increased, the islet cells are stimulated to produce an adequate amount of insulin to reduce the blood sugar to its normal level."

If our results on O_2 capacity and hemoglobin concentration may be interpreted to mean that dehydration of the blood is taking place, then this could be due to an increase in the molecular concentration within the tissue cells or to some change in the capillary walls. If the former were true then the increase in O_2 consumption (10) which occurs at this time might result in compounds which are retained within the cell for, as Macleod (8) suggests, "inspired oxygen is locked away in some form in the tissues so that its tension in the blood supply in the brain is inadequate" at the time of insulin convulsions.

SUMMARY

1. There is a slight fall in oxygen saturation of arterial blood in decerebrate cats preceding insulin convulsions, in spite of markedly increased ventilation. This could not be demonstrated in rabbits.

2. The hemoglobin concentration and O_2 capacity, however, rise sharply two to three hours after insulin in decerebrate and decapitate cats. Rabbits on the contrary usually show a slight fall.

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SOME FACTORS INFLUENCING THE ASSAY OF INSULIN

N. R. BLATHERWICK, M. LOUISA LONG, MARION BELL, L. C. MAXWELL AND ELSIE HILL

From the Chemical Laboratory of the Potter Metabolic Clinic, Santa Babara Cottage Hospital, Santa Barbara

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The rabbit is now universally used for testing the potency of insulin preparations. Any one who has used this method realizes that there is considerable variation in the response of individual animals. Manufacturers of insulin chiefly overcome this variability by using large numbers of animals but such a procedure is, obviously, impossible of application by laboratories preparing insulin in smaller amounts. It therefore becomes essential for the average worker to understand and control, insofar as possible, those factors which cause variations in response to insulin. The object of this paper is to record certain observations which have accumulated in this laboratory during a period of more than eighteen months. It is believed that our findings will be of service to others.

The influence of diet. Page (1) has reported that rabbits fed an acid-forming diet are more resistant to insulin than are those fed a base-forming diet. The increased resistance of the animals fed an acid-forming diet is ascribed by this author to a corresponding shift in the acid-base balance of the body. It seemed that the results obtained by Page might possibly have been due to a higher content of body carbohydrate, particularly glycogen, since the acid-forming diet used was also high in carbohydrate. McCormick, Macleod, Noble and O'Brien (2) have demonstrated that animals with larger amounts of glycogen in their tissues are, in fact, more resistant to insulin than are glycogen-poor animals.

It was noted early in our work that the response of rabbits to insulin varied greatly when the animals were taken directly from the feeding pens. At that time they were receiving a mixed diet of alfalfa hay and crushed barley. In order to standardize conditions we then fed our rabbits a diet of alfalfa hay only and fasted them for twenty-four hours before the test. This simple diet was purposely selected as being one low in carbohydrate and, therefore, a poor glycogen-former.[1]

Young Belgian hares which had never received insulin were selected for the particular experiments herein reported. It was planned to give these

[1] This diet was suggested by Dr. W. D. Sansum.

animals certain diets and to administer intravenously every seven days a fixed amount of insulin per kilogram of body weight. This regime was carried on until the animals had approximately doubled in weight. In case an animal experienced no convulsion after a certain dose a further amount was afterward given to produce this result. All rabbits were fasted twenty-four hours before the test. Those animals which received the alfalfa hay diet were also fed crushed barley on the experimental day after having had

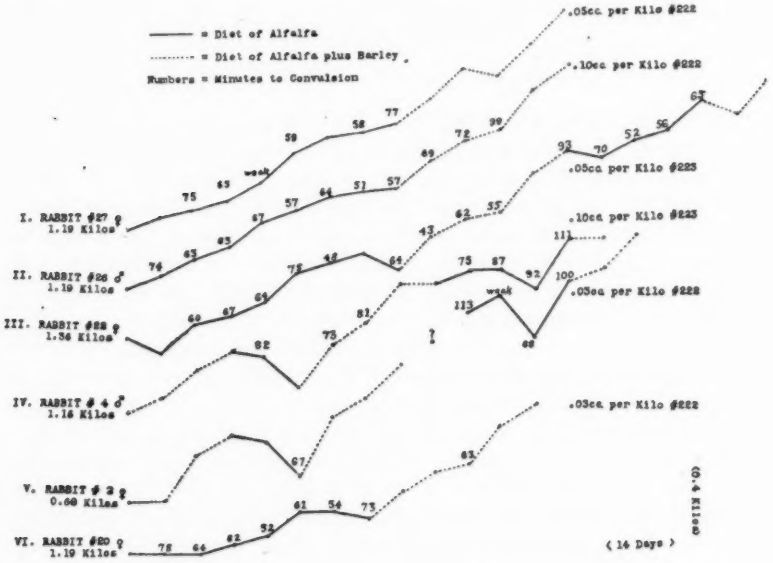

Fig. 1. I. This rabbit shows the "education" phenomenon. Convulsions quite regularly appeared on a diet of alfalfa hay but were lacking when barley was added. Satisfactory gains in weight.

II. This rabit shows increased resistance to first dose. Thereafter convulsions were produced on a diet of alfalfa hay the addition of barley resulted in an increasing time to convulsions with an ultimate failure to appear. Satisfactory gains in weight.

III. This rabbit illustrates the "education" phenomenon. There are evidences of greater resistance when barley was added to the alfalfa hay diet. Satisfactory gains in weight.

IV. A greater resistance is shown on the diet of alfalfa hay and barley. Gains in weight fairly satisfactory.

V. This rabbit never experienced convulsions on the diet of alfalfa hay and barley. After gaining 2.8 times the original weight, the same dose per kilogram produced convulsions. This was just about the convulsion dose for this animal.

VI. This rabbit shows greater resistance to the first dose. Convulsions regularly appeared on the diet of alfalfa hay but were lacking with one exception when barley was added to the diet. This was about the minimum convulsion dose. The gains in weight on the alfalfa diet were not satisfactory.

convulsions and on the following day. This addition of barley was for the purpose of replenishing the carbohydrate stores of the body.

Examination of curves I to VI (fig. 1) shows that convulsions are much easier to produce in rabbits which eat the base-forming diet of alfalfa hay than in those animals which are fed a mixed diet of alfalfa hay and crushed barley. These results confirm those reported by Page.

The greater resistance to insulin of rabbits fed the higher carbohydrate diet of alfalfa hay and barley is not due to a greater store of glycogen in the liver and in the muscles. This point was determined by subjecting young rabbits which had never received insulin to the following procedures.

GROUP	DIET	FASTED
I	Alfalfa and barley	No
II	Alfalfa	No
III	Alfalfa and barley	Yes
IV	Alfalfa	Yes

There were 4 animals in each group. Table 1 shows that rabbits fed a diet of alfalfa hay and barley have much larger amounts of glycogen in the liver and in the muscles but by withholding food for twenty-four hours the values for glycogen in these tissues reach approximately the same level as when the animals are fed a diet of alfalfa hay only and are fasted for twenty-four hours. It is seen that rabbits taken directly from the alfalfa feeding pens have only small amounts of glycogen in their tissues; the glycogen values show very little change when fasting is superimposed on this diet. These observations seem to eliminate glycogen as being a factor in producing the greater resistance to insulin of rabbits fed a high carbohydrate, acid-forming diet. The explanation offered by Page is probably correct. It would be interesting to determine in what manner a high carbohydrate, base-forming diet would affect the response to insulin.

The dosage of insulin. Some have believed that the dosage of insulin varies as the square of the body weight and not directly with the weight (3). In other words, they believed that the amount of insulin necessary to produce a convulsion in a 2 kgm. rabbit is not twice the amount required in a 1 kgm. rabbit but is instead 4 times that amount. In this laboratory we have always assumed that the dosage varied directly with the body weight and this has worked well in practice.

The figure shows that rabbits fed a standard diet of alfalfa hay and fasted for twenty-four hours before being used to test insulin react to the same dose per kilogram of body weight after they have doubled or more than doubled in weight. Our results, therefore, offer no support to the hypothesis that the dosage varies as the square of the body weight. On the other hand, they indicate that the amount required to produce a given result, namely, a convulsion, varies directly with the weight.

Examination of the curves reveals an interesting phenomenon; an "education" of rabbits to insulin. Rabbits eating a standard diet become more susceptible to insulin convulsions after they have experienced one or two or three convulsions. This phenomenon is so pronounced that we always produce convulsions in our new rabbits before they are used for any accurate work.

The care of rabbits. It may be helpful to record the care our rabbits are given. Young rabbits are started on insulin tests when they weigh about 1 kgm. They are first given sufficient insulin to produce convulsions in order to obtain the "education" phenomenon and are then used every seven days thereafter. We think it is important that the animals should have convulsions each time they are used. If any fail to do so after a given dose more insulin is injected to produce this result. Our rabbits are given convulsions every seven days even though there is no special preparation to be tested. Such a procedure certainly tends to stabilize

TABLE 1

Glycogen in tissues of rabbits

GROUP	PER CENT GLYCOGEN						TREATMENT
	Liver			Muscle			
	Minimum	Maximum	Average	Minimum	Maximum	Average	
I	5.88	7.92	6.99	0.20	0.38	0.32	Alfalfa and barley. Not fasting
II	0.00	0.26	0.11	0.07	0.29	0.16	Alfalfa. Not fasting
III	0.00	0.21	0.09	0.07	0.27	0.16	Alfalfa and barley. Fasting
IV	0.07	0.41	0.19	0.10	0.23	0.14	Alfalfa. Fasting

the experimental conditions. After the animals have experienced convulsions and have been anti-doted with glucose, they are returned to the cages and are fed alfalfa hay and crushed barley that and the following day. Young rabbits may require a third day on barley. After this, the diet consists of alfalfa hay only. A complete 24-hour fast precedes the test. The alfalfa hay must contain a goodly proportion of leaves to be satisfactory. Young animals should not be used for assaying insulin unless they are gaining in weight and mature animals should maintain their weights. It is highly important to keep weight records of all rabbits. In the light of our experience, it is believed that rabbits fed a diet of alfalfa hay and subjected to a fast of twenty-four hours before the tests, show less variation in response to insulin than do animals which eat a mixed diet of alfalfa hay and crushed barley. The insulin is always given intravenously, and blood samples are taken from the marginal ear veins before and one hour after the injections. The maximum hypoglycemic reaction occurs

about one hour after insulin is given intravenously. The drop in blood sugar and the production of convulsions are both considered in evaluating the potency of insulin preparations.

SUMMARY

The data presented show that rabbits fed the low carbohydrate, base-forming diet of alfalfa hay are less resistant to insulin than are rabbits fed the high carbohydrate, acid-forming diet of alfalfa hay and crushed barley. The greater resistance of animals fed the latter diet does not appear to be due to an increase in the glycogen content of the tissues, since animals maintained on both diets and fasted for twenty-four hours had practically the same amounts of glycogen in the liver and in the muscles.

The experiments cited indicate that the intravenous dosage of insulin in rabbits varies directly with the body weight.

Attention was called to the fact that insulin convulsions are more easily produced after the animals have experienced one or more convulsions.

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THE INFLUENCE OF ASPHYXIA ON VESTIBULAR NYSTAGMUS AND AN ATTEMPT TO LOCALIZE THE SEAT OF THIS INFLUENCE IN THE REFLEX ARC

N. KLEITMAN[1] AND A. DE KLEIJN

From the Pharmacological Institute of the University of Utrecht

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Some years ago it was observed that during asphyxia there was a reversal of the deviation in caloric nystagmus in rabbits (1). The present investigation was undertaken in order to study the asphyxial phenomena in greater detail and, if possible, to determine on what part of the reflex arc the action was exerted. The elements that had to be considered in this connection were *a*, the endings of the eighth cranial nerve in the labyrinth; *b*, the vestibular nuclei in the medulla; *c*, the nuclei of the motor nerves to the eye-muscles, and finally *d*, the endings of these nerves in the muscles and perhpas the muscles themselves.

METHODS. We employed rabbits for all our experiments. The animals were anesthesized with ether, and a tracheal cannula inserted. The skull-cap was entirely removed, and the cerebral hemispheres extirpated, leaving the thalamus intact (Thalamustier of Magnus). In the rabbit this can be done without any bleeding, especially if the carotid arteries are occluded during the operation. This permitted us to dispense with the further administration of ether. In some control cases, however, the cerebrum was left intact and the ether anesthesia continued till the end of the experiment. Care was taken not to make the anesthesia too deep, as in that case nystagmus as well as the other reflexes disappear. To register the contractions of the eye-muscles we used the method of Topolanski-Bartels (2), (3). The external and internal recti muscles of one eye were isolated in the following manner. The sclera was cut close to the margin of the cornea, and by pulling on the bulbus the eye-muscles were brought into view. The two muscles in question being single, they were easily distinguished from the superior and inferior recti, which are accompanied by the oblique muscles. After the insertions were severed from the eye-ball, the free ends of the muscles were connected by threads to levers which recorded their contractions on a kymograph. The other muscles were then cut, and the eye-ball extirpated. Here, too, bleeding could be avoided by temporarily clamping the carotid on that side. The

[1] Fellow in Physiology of the National Research Council.

writing levers were constructed on the principle of the Keith Lucas lever, both levers being made to slide on the same vertical wire and thus insuring registration in the same ordinate. By the use of jewel bearings such levers can be made very delicate. To elicit nystagmus one ear was irrigated with cold water (15°C.) the reservoir being 1 meter above the ear. After we convinced ourselves that exactly the same asphyxial effects could be obtained by syringing one ear with cold water or the other ear with warm water (40°C.), we adopted the cold water irrigation for its greater simplicity. Nystagmus could also be produced by unilateral labyrinth extirpation, or by bilateral extirpation, if the two labyrinths were destroyed a few days apart. In the latter case we have the so-called Bechterew nystagmus (4). In several animals the blood pressure was also recorded. The vagi were either cut or left intact, but no difference in

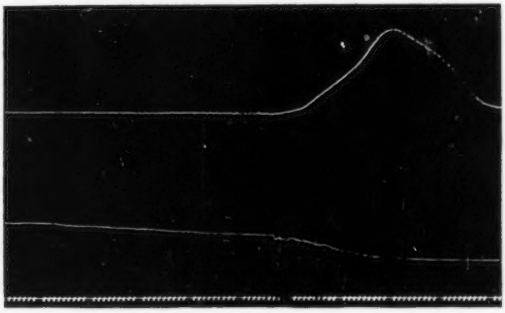

Fig. 1. Rabbit 27. Ether anesthesia. Carotids tied, vagi cut. Left eye prepared. Upper tracing is from rectus internus, middle tracing from rectus externus, lower tracing gives time in seconds and 10 seconds. Asphyxia resulted in a contraction of the rectus internus and a simultaneous relaxation of the rectus externus.

the course of the events was observed. The asphyxia was produced by merely clamping the rubber tube at the end of the tracheal cannula.

Results. The results are based on 46 successful experiments.

In the first place we shall describe the phenomena that occur during asphyxia in a thalamus-rabbit or a normal anesthetized rabbit, with the eye-muscles at rest. A short time after the closing of the tracheal cannula (one to three minutes), at the moment when the blood pressure, after a preliminary rise, has fallen to a low level, there occurs a very strong contraction of one muscle and a simultaneous relaxation of its antagonist, which means a deviation of the eye to one side (fig. 1). In many cases, however, this is preceded by a slight deviation of the eye in the opposite direction. In other words, the record would show first a slight contraction of the rectus internus and a relaxation of the rectus externus, followed by a strong contraction of the rectus externus and a

relaxation of the rectus internus, or vice versa. There was no regularity as to which muscle contracted and which relaxed. Later on we shall try to account for this varied behavior of the two muscles.

We now observed the effect of asphyxia on the eye-muscles during nystagmus induced by continuous irrigation of one ear with cold water. As is shown in figure 2, syringing the left ear with cold water causes the rectus externus of the left eye to contract and the rectus internus to relax. The deviation is to the left, and the quick component of the nystagmus to the right. As a result of asphyxia the rectus externus, or the contracted muscle, relaxes and the rectus internus contracts. In the majority of cases the immediate effect of asphyxia was a stimulation of the nystagmus until the quick component entirely disappeared, the contracted muscle remained contracted and the relaxed muscle relaxed. This was followed by a reversal of the deviation, the contracted muscle now relaxing and the relaxed muscle contracting (fig. 3). In a few instances not only was the deviation reversed, but we could also observe some jerks in the direction opposite to that of the original caloric nystagmus. At this stage the animal could still be revived by instituting artificial respiration, spontaneous respiration returning in a few minutes, and asphyxia could thus be produced several times in the same animal. We usually irrigated the other ear, eliciting nystagmus in the opposite direction, and asphyxiating the animal obtained the same result but with the rôles of the two muscles interchanged. In all cases the final maximal contraction was observed in the muscle which, in virtue of the particular nystagmus induced, was relaxed.

The same effects of asphyxia as those observed during the nystagmus produced by syringing one ear with cold water could be seen during the spontaneous nystagmus often resulting from ether anesthesia. This type of spontaneous vestibular nystagmus appears not only in normal animals, but also in those in whom both labyrinths had been extirpated. In all experiments with asphyxia, with the eyes at rest or in action, if the other intact eye was observed, it could be seen to be deviated in the same direction as the prepared eye. If one eye was deviated to the right, the other was deviated to the right also. In one case, using a normal unanesthetized animal and recording the movements of both intact eyes (by connecting the cocainized cornea by means of a thread to the writing lever), we accidentally injected an overdose of a drug whose action we were studying, thus killing the animal. We obtained a fine asphyxia record showing that both eyes were deviated in the same direction, in this case both to the left. We then performed an experiment where we registered the contractions of both external and both internal recti muscles (using two kymographs). Inducing caloric nystagmus, we then asphyxiated the animals and could see that if the terminal maximal contraction

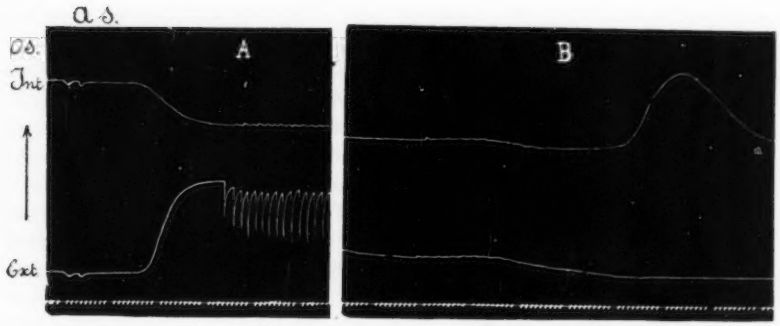

Fig. 2. Rabbit 24. Ether anesthesia. Carotids tied, vagi cut. Left eye prepared. Upper tracing is from rectus internus, middle tracing from rectus externus, lower tracing gives time in seconds and 10 seconds. *A* shows the result of syringing the left ear with cold water. The rectus internus is relaxed, the rectus externus contracted, and there is established a nystagmus to the right. *B* shows the two muscle 2 minutes after the beginning of asphyxia, the ear being continuously irrigated. The originally contracted muscle, the rectus externus relaxes, and the relaxed muscle, the rectus internus, contracts.

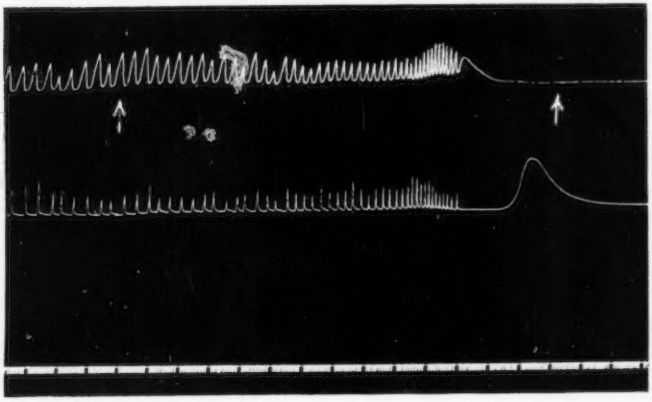

Fig. 3. Rabbit 6. Ether anesthesia. Carotids and vagi intact. Upper tracing is from rectus internus of the left eye, middle tracing from rectus externus of the same eye; lower tracing gives time in seconds and 10 seconds. At the first arrow the tracheal cannula was occluded, at the second arrow artificial respiration was instituted. At first there is a stimulation of the caloric nystagmus maintained by syringing the right ear with cold water, the quick component disappears, the contracted muscle remaining contracted. This is followed by a relaxation of the contracted muscle (rectus internus) and by a contraction of the relaxed muscle (rectus externus).

took place in the rectus externus of one eye it also took place in the rectus internus of the other eye, showing that both eyes were deviated in the same direction.

These phenomena, we thought, indicated that the effect of asphyxia was exerted on the medullary centers rather than on the nuclei of the eye-muscles, as otherwise it would be difficult to explain why one rectus internus relaxed and the other contracted, but to establish it firmly, and especially to see what part is played by the nerve endings, we investigated each part of the reflex arc separately. The method we employed can be utilized in a general way in any study on vestibular nystagmus, especially where the action of drugs is concerned, this being practically an untouched field.

The effector end of the arc. That asphyxia has no effect on the eye-muscles themselves could be shown by curarizing an animal to a degree when even the respiratory muscles were paralyzed. In such experiments the carotid on the corresponding side was not tied, as Wessely has shown that the eye-muscles depend for their blood supply mainly upon the carotids (5). Asphyxia was produced by discontinuing the artificial respiration, but no effect on the muscles could be observed. Intravenous injection of "panitrin" (papaverine nitrite) which acts on the eye-muscles themselves, under these conditions produced a contraction of both muscles, indicating that the muscles were in good condition and that their circulation was intact. This could also be shown by allowing the animal to recover from the curare effect and then producing asphyxia, which always resulted in a typical contraction of one muscle and a relaxation of the other.

To determine the possible effect of asphyxia on the endings of the motor nerves to the eye-muscles the oculomotor nerve was cut intracranially which in a thalamus rabbit is done by lifting the front part of the brain stem and exposing the nerve, and then snipping it with scissors. Asphyxia then had the usual effect on the rectus externus, but none on the rectus internus, thus showing that the asphyxial contraction of the muscles was in no way due to action on the nerve-endings. Intravenous injection of 0.25 to 0.4 mgm. nicotine per kilo produced a contraction of both muscles, proving that the circulation of the denervated muscle was not impaired.

The nuclei of the cranial nerves. To see if asphyxia had any effect on the nuclei of the motor nerves to the eye-muscles, we proceeded as follows. Having prepared thalamus rabbits and irrigated each ear in turn with cold water we could make sure that both muscles reacted to stimulation in a normal manner. We then cut through the brain stem between the nuclei of the third and sixth nerves, usually at the posterior margin of the anterior corpora quadrigemina, deep enough to cut the posterior

longitudinal fasciculus. After this operation, on syringing either ear, one should obtain a response only from the rectus externus. After the experiment the brain stem was always removed, hardened in formalin, and examined later on. In only two cases could we obtain a reaction from both muscles after section of the brain stem, but careful histological examination, for which we are indebted to Doctor Rademaker, revealed in each case that the posterior longitudinal fasciculus was not cut through. In later experiments we therefore cut through the entire brain stem, but tried to spare the vessels at the base. In all cases, where subsequent histological study showed the section to have been made in the right place, we could get caloric nystagmus only from the rectus externus. Producing asphyxia in such an animal, whether in the presence or in the absence of caloric nystagmus, we would get the usual effects from the rectus externus. To show that the absence of a reaction from the rectus internus was not due to an interruption of the circulation to the nucleus of the third nerve, we always injected intravenously at the end of the experiment a dose of nicotine which had been shown to act alone on the nuclei of the motor nerves to the eye-muscles when the carotids are tied (6). The contraction of the rectus internus after such an injection showed that the circulation was intact (fig. 4). It thus appears that the motor phenomena accompanying asphyxia cannot be ascribed to the stimulation of the nuclei of the third and sixth nerves.

The receptor end of the arc and the vestibular nuclei. The only two elements of the reflex arc that could now be held responsible for the asphyxial effects were the sensory endings of the eighth nerve in the inner ear and the nuclei of the vestibular system in the medulla. That the endings of the eighth nerve were not *necessary* for the phenomena above described could be demonstrated in the following way. Nystagmus that appears after the unilateral labyrinth extirpation in the rabbit lasts but a few days. If the remaining labyrinth is now destroyed a typical vestibular nystagmus is produced, commonly known as Bechterew nystagmus. Such animals were prepared for eye-muscle registration in the usual way, and on asphyxiating them we always obtained the same results as when the labyrinths were intact and the nystagmus was caloric in origin. If both labyrinths are extirpated simultaneously, there is no nystagmus at all, nor can it be elicited by irrigating one or the other ear with cold water. Asphyxiating the animal, we could obtain the usual contraction of one muscle and relaxation of the other, as when we asphyxiated a normal animal with the eye-muscles at rest. Whether in the normal animal the labyrinths do not coöperate with the vestibular nuclei in the production of the asphyxial effects could not be determined.

Discussion. From the results of our experiments we can conclude that the nuclei of the motor nerves and their endings in the muscles are

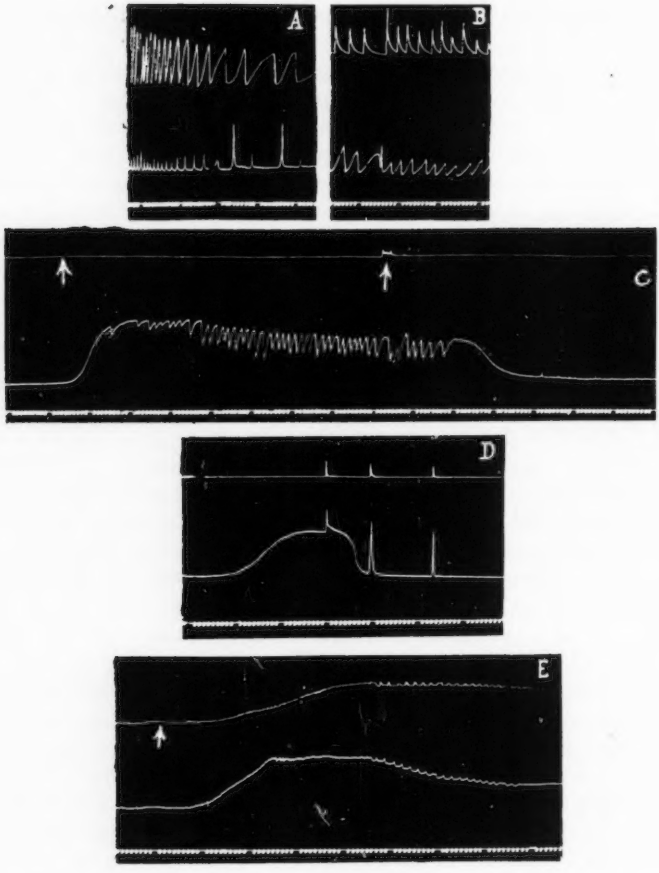

Fig. 4. Rabbit 33. "Thalamustier." Carotids tied, vagi intact. Left eye prepared. In each record the upper tracing is from the rectus internus, middle tracing from the rectus externus, lower tracing gives time in seconds and 10 seconds. *A* represents nystagmus resulting from the irrigation of the right ear with cold water, and *B* shows nystagmus in the opposite direction, when the left ear is syringed with cold water. In *C* at the first arrow the left ear was syringed, producing a contraction of the rectus externus, and at the second arrow the right ear was syringed, producing a relaxation of the rectus externus. The brain stem had been previously cut between the nuclei of the third and sixth nerves, and it will be observed that syringing either ear had no effect on the rectus internus. The effect of asphyxia is shown in *D*. There is a contraction of the external muscle, but no effect on the internal muscle, the three peaks in each tracing being due to terminal respiratory movements mechanically affecting the writing levers. After the animal was revived injection of 2 mgm. nicotine per kilo (intravenously) produced a contraction of each muscle, as shown in *E*, indicating that the blood supply of the nucleus of the third nerve was intact.

not affected by asphyxia. The motor phenomena accompanying asphyxia, the stimulation of the nystagmus and the final deviation of the eyes, probably have their origin in the medulla, and if the labyrinths are also affected, the action exerted by them adds itself algebraically to that exerted by the medullary centers. As to the cause of these phenomena the following explanation suggests itself. Asphyxia, as is known, produces a stimulation of the cells in the medulla, followed by a paralysis. If nystagmus is present at the time the asphyxia is produced, i.e., if the cells of the vestibular system producing the nystagmus are in a state of excitation, then the stimulation resulting from the asphyxia accentuates that nystagmus, the result being stronger and more frequent contractions of the muscles, followed by a deviation without the quick component. This vestibular system becomes fatigued and is paralyzed before the system of cells which produces nystagmus in the opposite direction, and the unopposed action of this latter system, which had not been fatigued by previous activity, should result in a nystagmus opposite to the original one. That only seldom could such a reversal of the nystagmus be observed and that in most cases we could notice only a deviation in the opposite direction, i.e., a strong contraction of the originally relaxed muscle, may be due to the impaired condition of the cells on account of a lack of oxygen or accumulation of carbon dioxide, as at this point the blood pressure is practically down to the base line. It is known that in very deep anesthesia and under the influence of different narcotics syringing the ear will produce only a deviation of the eyes, the quick component of the nystagmus being absent. It is surprising, however, that the terminal contraction should be so powerful.

The same argument can be used in the attempt to explain the effects of asphyxia when the eye muscles are at rest. In most animals the two opposing vestibular systems are not balanced, as can be shown by the difference in the responses obtained by turning the animal in one or the other direction the same number of times and at exactly the same speed. Under these circumstances the same sequence of events should be expected whether the asphyxia is induced during nystagmus or with the eye-muscles at rest. In fact, asphyxia produces a deviation of both eyes sometimes in one direction, sometimes in the other.

SUMMARY

1. Asphyxia produces a deviation of both eyes in the same direction, either both to the right or both to the left. If the contractions of the isolated internal and external recti muscles are recorded, it is seen that one muscle contracts strongly and its antagonist relaxes.

2. If asphyxia is produced during vestibular nystagmus, of whatever origin, the nystagmus is first stimulated until a deviation without the quick component is obtained, and then there is a reversal of the deviation.

3. By a method which is applicable in a general way to the analysis of the action of any agent that affects vestibular nystagmus it was shown that the motor eye phenomena resulting from asphyxia are probably of medullary origin.

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MINERAL METABOLISM STUDIES WITH DAIRY CATTLE[1]

H. G. MILLER, P. M. BRANDT AND R. C. JONES

From the Department of Agricultural Chemistry and the Department of Dairy Husbandry, Oregon Experiment Station, Corvallis

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Investigations which have shown the ability of the lactating animal to maintain milk yield during which time the body supply of calcium and phosphorus is being depleted and no pathological symptoms are apparent, have suggested the importance of studies pertaining to the mineral balance prevailing in these animals during the reproductive cycle. The failure of Forbes and co-workers (1) to prevent the loss of calcium and phosphorus from the body during early lactation when the dairy rations were supplemented with calcium salts and his later observation that these animals stored calcium and phosphorus during low production and the dry period, have led this investigator to emphasize the importance of a long dry period in order to build up the mineral supply of the body. Hart and associates (2) were successful in obtaining a calcium and phosphorus storage during heavy lactation and have suggested the presence of certain organic constituents in green feeds or carefully cured hay which aid in mineral assimilation.

In recent work (3) they have also shown that the state of mineral depletion is an important factor in favoring mineral storage under ideal feeding conditions for mineral assimilation, namely, green feed and a liberal supply of calcium and phosphorus. In the work to be reported here the experimental procedure was practically the same as above and the results obtained, while not entirely of the same order due to differences in feeding procedures, are in accordance with the results of the latter investigators.

Experimental. Three registered Holstein cows were used in this experiment. No. 216 was seven years old and in the eighteenth day of the lactation period; no. 218 was six years of age and in the twenty-third day of lactation, and no. 222 was four years of age and in the fifteenth day of the lactation period. They were given a ration daily consisting of ten pounds of red clover hay, thirty pounds of oats and vetch silage, and approximately one pound of grain (corn fifty-eight, bran thirty-two, oil meal ten) to four pounds of milk produced. After a three-week period

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TABLE 1

Average daily mineral balance animal 216

PERIOD	AVERAGE DAILY MILK YIELD	NITROGEN	SULFUR	PHOSPHORUS	CHLORINE	CALCIUM	MAGNESIUM	POTASSIUM	SODIUM
		FEED	FEED	FEED	FEED	FEED	FEED	FEED	FEED
		MILK	MILK	MILK	MILK	MILK	MILK	MILK	MILK
		URINE	URINE	URINE	URINE	URINE	URINE	URINE	URINE
		FECES	FECES	FECES	FECES	FECES	FECES	FECES	FECES
		BALANCE	BALANCE	BALANCE	BALANCE	BALANCE	BALANCE	BALANCE	BALANCE
	kilos	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
January 15 to February 5	19.55	208.4	17.0	47.7	36.9	72.9	32.1	138.4	18.7
21 days on basal ration		82.4	5.2	21.5	16.0	22.1	2.0	23.3	6.1
		58.7	2.2	0.2	6.6	0.2	4.2	102.0	7.1
		90.8	8.5	31.0	15.5	56.0	24.1	10.7	3.7
		−23.5	+1.1	−5.0	−1.2	−5.4	+1.8	+2.4	+1.8
February 5 to 26, 21 days,	19.04	219.0	18.2	74.6	39.1	117.5	34.4	143.2	19.7
basal ration plus 150		76.4	4.9	19.6	16.5	20.7	2.1	21.0	5.3
grams bone meal daily		56.0	2.2	0.3	7.0	0.0	4.5	111.0	10.0
		99.3	9.0	49.3	17.0	93.0	26.0	9.3	3.4
		−12.7	+2.1	+5.4	−1.4	+3.8	+1.8	+1.9	+1.0

TABLE 2

Average daily mineral balance animal 218

PERIOD	AVERAGE DAILY MILK YIELD	NITROGEN	SULFUR	PHOSPHORUS	CHLORINE	CALCIUM	MAGNESIUM	POTASSIUM	SODIUM
		FEED	FEED	FEED	FEED	FEED	FEED	FEED	FEED
		MILK	MILK	MILK	MILK	MILK	MILK	MILK	MILK
		URINE	URINE	URINE	URINE	URINE	URINE	URINE	URINE
		FECES	FECES	FECES	FECES	FECES	FECES	FECES	FECES
		BALANCE	BALANCE	BALANCE	BALANCE	BALANCE	BALANCE	BALANCE	BALANCE
	kilos	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
January 15 to February 5 21 days, basal ration	20.64	225.	18.3	52.97	42.0	73.3	34.4	142.2	21.9
		83.4	5.3	22.07	14.6	22.8	2.4	24.8	5.8
		64.7	2.6	0.16	14.2	0.4	5.1	95.9	9.6
		99.9	9.4	38.05	13.4	57.5	24.7	15.5	4.0
		−23.0	+1.0	−7.31	−0.2	−7.4	+2.2	+6.0	+2.5
February 5 to 26, 21 days, basal ration plus 9.07 kilos green kale daily	23.29	275.3	26.9	60.37	50.5	94.1	39.0	178.3	22.4
		91.0	5.8	23.05	17.9	24.2	2.9	24.9	5.7
		74.6	6.3	0.23	18.0	0.0	5.9	142.6	15.5
		111.4	10.8	37.05	16.0	73.9	28.3	11.0	3.8
		−1.7	+4.0	+0.04	−1.4	−4.0	+1.9	−0.2	−2.6

TABLE 3

Average daily mineral balance animal 222

PERIOD	AVERAGE DAILY MILK YIELD	NITROGEN	SULFUR	PHOSPHORUS	CHLORINE	CALCIUM	MAGNESIUM	POTASSIUM	SODIUM
		FEED	FEED	FEED	FEED	FEED	FEED	FEED	FEED
		MILK	MILK	MILK	MILK	MILK	MILK	MILK	MILK
		URINE	URINE	URINE	URINE	URINE	URINE	URINE	URINE
		FECES	FECES	FECES	FECES	FECES	FECES	FECES	FECES
		BALANCE	BALANCE	BALANCE	BALANCE	BALANCE	BALANCE	BALANCE	BALANCE
	kilos	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
January 30 to February 12, 13 days on basal ration	18.93	204.1	16.6	46.6	38.3	71.9	31.6	134.8	18.7
		81.5	5.3	19.7	13.7	22.5	2.3	20.9	5.5
		53.7	1.8	0.2	7.8	0.4	4.4	94.0	5.2
		84.0	7.6	31.1	13.6	55.0	21.6	11.6	2.6
		−15.1	−1.9	−4.4	+3.2	−6.0	+3.3	+8.3	+5.4
February 12 to 26, 14 days, basal ration plus 150 grams bone meal daily	19.83	209.6	17.4	71.5	37.6	118.3	33.2	140.8	18.9
		77.3	5.3	18.2	15.9	21.8	2.5	22.0	5.4
		58.0	2.1	1.0	9.0	0.1	4.6	108.0	7.1
		−15.9	+1.3	+6.5	−3.5	+5.8	+2.1	−1.9	−1.4

on the above ration, the ration of animal 216 was supplemented daily with 150 grams of bone meal. Cow 222 received the same rations except the periods were of two weeks duration. The ration of cow 218 was supplemented with twenty pounds of kale during the second period of three weeks. The animals received daily a definite quantity of salt in their grain. The mineral intake through the water consumed was determined by weighing and analyzing the water. At the beginning and end of the experimental periods the cows weighed as follows: no. 216, 1180 pounds and 1118 pounds, no. 218, 1196 pounds and 1137 pounds, and no. 222, 1055 pounds and 1041 pounds.

During the entire period of six weeks the animals were confined to the metabolism stalls with a quantitative collection of excreta by attendants. A definite daily aliquot of feces, urine and milk was composited for a period of seven days and this sample used in the analysis for calcium, phosphorus, magnesium, potassium, sodium, chlorine and sulfur. For nitrogen, daily samples of urine and feces were taken and digested immediately with

TABLE 4

Calcium and inorganic phosphorus content of the blood

	MGM. Ca IN 100 CC. BLOOD		MGM. INORGANIC P PER 100 CC. BLOOD PLASMA	
	Period I	Period II	Period I	Period II
216	7.02	7.70	3.5	4.7
218	7.32	7.47	3.7	5.0
222	7.38	7.05	4.0	7.5

sulfuric acid. Chlorine was also determined on daily samples of the urine by the Volhard Arnold method. The dried feces were used for the remaining analysis and representative samples of each feed were taken for analysis.

The calcium and magnesium were determined by the method of McCurdden. The feeds and feces were ashed by ignition and the urine and milk by the sulfuric acid-nitric acid method. Phosphorus in feces and feeds was determined by ashing with magnesium nitrate, with urine and milk, the Neumann method was followed. Potassium and sodium were weighed as the chlorides after precipitating the phosphates, sulfates and magnesium with excess of barium hydroxide solution. Excess of barium and the calcium were removed with ammonium carbonate. Potassium was finally weighed as the chlorplatinate. Sulfur was determined by the sodium carbonate-sodium peroxide fusion, except in the case of the urine, where the Denis modification of Benedict's method was used. Chlorine except as mentioned above was determined by ashing at a low red heat with sodium carbonate and then following the Volhard Arnold procedure.

In the latter part of each of the two periods samples of blood from each animal were obtained and the inorganic phosphate in the plasma was determined according to the method of Marriott and Haessler (4) and the calcium of the whole blood by the method of Long and Bushill (5).

Discussion. We realize that our experimental periods were too limited in time to warrant any far-reaching conclusions. Between periods no interval of time was allowed for readjustment to the change in the ration. However, the change in the quality and quantity of the ration was such that the animal readily adapted itself as far as consumption was concerned. Furthermore this mineral balance study included not only calcium and phosphorus but other elements as potassium and sodium. While there is a delay in the excretion of calcium and phosphorus due to the time the feed residues remain in the alimentary tract, variation in the solubility and eliminative processes of these two groups of elements permits the potassium and sodium to be excreted more rapidly.

Throughout the experimental period the animals were in a negative nitrogen balance. The animal whose nitrogen intake was increased by supplementing the basal ration with kale did not pass into a positive balance. There was, however, a decrease in the negative balance accompanied by a marked increased nitrogen metabolism as shown by the increased milk production and increased urinary nitrogen.

Contrary to the results of Forbes, the feeding of bone meal brought about increased calcium and phosphorus retention so that negative balances during the first period were changed into positive balances during the second period. A greater mineral depletion of the body supply may have been the determining factor in causing calcium and phosphorus storage in this experiment as Hart and associates (3) have recently observed in their work. No special precautions were taken in the curing of the clover hay and there is no reason to believe that the vitamin influence was any greater than ordinarily found in feeding red clover hay. Other investigators have observed similar results. Fingerling (6) changed negative calcium and phosphorus balances into positive balances by adding calcium phosphate to the rations of milking goats. Later Hunt (7) observed calcium retention where calcium phosphate was given to goats during lactation.

The changing of a negative phosphorus balance to one of maintaining a phosphorus equilibrium through the feeding of green kale is certainly noticeable. This apparently is due to some specific effect of the green feed as examination of the data shows that it cannot be explained entirely on the basis of increased phosphorus intake. In comparing the digestibility of phosphorus during the two periods it is observed that during the kale period 38.6 per cent of the phosphorus was absorbed from the intestine compared to 28.1 per cent during the first period. Though there

was greater calcium assimilation a positive calcium balance was not obtained through the feeding of this green feed; perhaps the absence of a calcium supplement did not produce the ideal conditions for calcium absorption as reported by previous workers (3). The greater absorption of calcium could be accounted for on the increased plane of calcium intake and the general increase in metabolism. Introducing kale in the ration increased and maintained a higher milk yield. From our observations kale is superior to green pasture or silage in causing increased milk yield. We have just observed the same result with this same animal after she has been producing heavily for about a year without being bred.

The feeding of one pound of salt to one hundred pounds of grain did not appear sufficient to maintain chlorine equilibrium. Increasing the grain salt mixture probably would have resulted in more satisfactory chlorine and nitrogen balances. Considering the total balance for the entire experimental period there was no loss of potassium and sodium from the body. Kale evidently caused a higher proportion of potassium to be absorbed from the alimentary tract but the increased urinary potassium led to a negative potassium balance. The sulfur balances were positive throughout, the interpretation of which is difficult, inasmuch as there was a loss in body weight accompanied by a negative nitrogen balance.

The calcium content of the whole blood taken from the cows at the two different periods was practically the same. This is in general accordance with the findings of other investigators (8) although certain workers (9) have found the calcium content to vary in the blood from lactating cows when the ration was changed. Unlike the calcium, the inorganic phosphate content of the plasma varies. This was demonstrated in the analysis reported here. During the second period when the animals were in phosphorus equilibrium or were storing phosphorus, the inorganic phosphate content of the plasma was increased.

SUMMARY

1. A quantitative study on the balance of nitrogen, sulfur, phosphorus, chlorine, calcium, magnesium, potassium and sodium was made with three liberally milking cows during the early lactation period on a ration of red clover hay, oats and vetch silage and grain. In a second period two cows received this basal ration plus bone meal and the third cow was given the basal ration plus kale.

2. Each of the cows lost significant quantities of calcium and phosphorus from their body supply when on the basal ration.

3. When bone meal was added to the ration the animals stored calcium and phosphorus so that negative balances were changed to positive.

4. Kale increased the milk yield and absorption of total solids from the intestine. Negative phosphorus balances were changed to one of phosphorus equilibrium and maintained accordingly while the kale was fed.

5. Though the animals were in a negative nitrogen balance throughout the experimental period, the sulfur balance was positive. The body supply of chlorine became less probably due to the insufficient quantity of sodium chloride added to the grain. Apparently for the total experimental period there was a storage of potassium, sodium and magnesium.

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OBSERVATIONS ON THE CIRCULATION DURING HYPOGLYCEMIA FROM LARGE DOSES OF INSULIN[1]

D. J. EDWARDS AND IRVINE H. PAGE

From the Physiological Laboratory, Cornell University Medical College, New York City

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The extensive work which has been done on the influence of insulin on the organism is so well known that any discussion of particular aspects of this subject is unnecessary. Suffice it to say that the literature contains few reports concerning the functional state of the circulatory system in hypoglycemia from insulin.

The condition of low blood sugar has been produced experimentally in a number of ways; e.g., by hepatectomy (1), by phlorhizin (2) and by hydrazin sulphate (3). In each of these procedures muscular weakness has been noted and in many instances characteristic nervous disturbances. It is probable that in each of these experimental hypoglycemias other functional changes are concerned which are specific and complex in character. But the common alteration in muscular activity and nervous control suggests a relation to the altered blood sugar level.

The recent observations of Klewitz (4) indicate that the concentration of sugar in the blood has little influence upon the muscular activity of the heart. The data presented by this observer show that the degree of utilization of sugar by a perfused heart bears practically no relation to the power of the beats. On the other hand there are the early observations of Locke and Rosenheim (5), showing that the addition of glucose to the perfusion fluid has an augmenting and sustaining effect; and later the studies of Evans (6) on the metabolism of the heart, in the heart-lung preparation, showing that about one-third of the energy supply comes from the oxidation of carbohydrate. These results suggest that the rôle of glucose for the isolated heart is one of increasing its activity and supplying much of its energy material.

In the early work with insulin Banting and co-workers (7) apparently did not regard as significant the changes in circulatory dynamics in hypoglycemia. They state, "While there is no evidence of marked cardiovascular disturbance in a typical convulsion (blood being usually readily

[1] A preliminary report of this work appeared in the Proc. Soc. Exp. Biol. and Med., 1923, xxi, 170.

obtained from the ear vein) this is sometimes present, making it very difficult to obtain any blood." In some later investigations, changes have been noted that are suggestive of involvement of the circulatory system. Fletcher and Campbell (8) call attention to the appearance in some instances of an increase in pulse rate and a fall in blood pressure, and Geyelin and co-workers (9) use the term "insulin shock" to designate a set of symptoms some of which are indicative of the beginning of circulatory failure. The results of Collip (10) on the alkaline reserve of the blood are significant in that he was able to show a reduced CO_2 combining power following the injection of large doses of insulin. In confirmation of this suggestion of an acidosis he was able further to show an increase in the ketone bodies of the urine.

In analyzing further the evidence presented in the investigations cited on the sugar utilization of the heart, the signs of muscular weakness in hypoglycemia, and the symptoms, under excessive doses of insulin, of circulatory disturbance and of acidosis, we were impressed with the possibility of some degree of circulatory failure as a contributing factor in the complex of insulin toxemia. The present experiments were undertaken for the purpose of studying more fully the functional behavior of the cardio-vascular system during the condition of low blood sugar concentration from large doses of insulin.

Methods. Dogs of varying size and age were used in these experiments. Their state of nutrition also varied somewhat as they were generally taken for the experiments a few days after being brought to the laboratory. In no case, however, was an animal used that exhibited distinct signs of lowered physical vigor from under-nutrition.

Records of mean blood pressure from the femoral or carotid artery have been made at frequent intervals throughout the course of each experiment. In some experiments pressure pulse tracings have been taken by using a form of optically recording membrane manometer (11), and in other instances the same recording device has been used to follow the pressure changes directly in the left ventricle. We have also employed the cardiometer method of studying the heart activity, using for this a photographically recording system. Heart sounds have been taken in some experiments by a direct recording method (12). Finally, the electrocardiogram has been taken in several instances in which the heart was not exposed to other direct experimental procedures.

The blood sugar determinations have been made by the Shaffer-Hartmann (13) method. The samples for these tests have been taken from the jugular vein, and an ordinary glass syringe which contained a few crystals of powdered oxalate was used for drawing the blood.

After performing the necessary operative work our procedure has been to take a set of records and a blood sample, which served as the index of

the normal state. The insulin was then given intravenously in a single large dose ranging from 20 to 36 units per kilo body weight. Cardiovascular records were then taken at intervals of about fifteen minutes and blood samples usually at the end of the first hour and at intervals of about thirty minutes thereafter.

Anesthesia. In these experiments it was necessary to have the animal insensitive to such operative steps as isolation of blood vessels and exposure of the heart. A general anesthesia, therefore, was required. Most of the ordinary laboratory anesthetics are known to influence the concentration of blood sugar, and often they markedly depress the circulatory system. In order to fulfill all of the requirements, therefore, it was necessary to obtain general anesthesia without disturbing blood sugar concentration or altering the function of the heart and blood vessels.

In some earlier experiments Page (14) has shown that the hypnotic iso-amyl-ethyl-barbituric acid has practically no effect on the blood sugar concentration. The condition of the circulation in animals under the influence of this drug, as shown by control experiments, has been very favorable, and we believe that, when administered in proper doses its disturbing influence is less than that from most other methods of anesthesia. A dose of the drug just sufficient to induce light sleep was usually enough to render the animal analgesic. In this condition it has been possible to conduct experiments for three to five hours with a well sustained blood pressure, with little change in heart rate, and with no apparent change in the dynamic capacity of the heart. The animal sleeps quietly when the dosage is right, and in this state, whenever touched, it exhibits a slow extensor movement of all of the extremities that is very suggestive of the reflex stretching act of a normal animal following sleep.

We have usually prepared the hypnotic for use by dissolving it in a hot aqueous solution to which just sufficient NaOH was added to render the drug soluble in a 10 per cent concentration. In our early experiments the drug was administered subcutaneously. The most effective dose, when given in this way, we found to be 70 mgm. per kilogram body weight, and an average period of about one hour was usually necessary for the development of analgesia. The comparatively long period required for the drug to act proved to be the main objection to this method of administering it. In later experiments we have been injecting the drug intraperitoneally and have obtained satisfactory analgesia in this way, usually with a dose of 50 mgm. per kilogram of body weight. Moreover, the time required for action was reduced to ten to twenty-five minutes.

It may be mentioned in passing that the method outlined above of giving this drug intraperitoneally in 50 mgm. doses per kilogram has been employed for the anesthesia in connection with some other experiments in which survival of the animal was required, and that the results have been entirely successful.

Blood sugar and alkaline reserve. The curves for the blood sugar concentration obtained from our data exhibit the same general course as those reported by other workers. There is, however, this feature of difference in our results, that the minimum level was not usually reached until two hours or more after the injection of the insulin. This fact may have an interesting bearing upon the mechanism for the disappearance of blood sugar. Macleod (15) puts forth the view that insulin lowers blood sugar partly through increasing the combustion process in the muscles. Our experimental animals were kept in practically complete physical repose by the hypnotic. The degree of metabolism, therefore, was probably somewhat less than it would be in an animal of similar size but not under the influence of a drug. A retarding of the process of sugar combustion may have been brought about in this manner.

We have also been able to corroborate the findings of Collip (10) with reference to the CO_2 combining power of the blood. The results in five experiments in which we have determined the alkaline reserve by the Van Slyke method show values ranging from 10 to 16 per cent below those taken on the same animal's blood at the beginning of the experiment.

Mean blood pressure and pulse form. Records of mean blood pressure have been taken in all of our experiments for the purpose of showing the directional trend of the cardio-vascular adjustment. The curves plotted from these results indicate two general types of reaction. In one group the mean blood pressure exhibits a gradual decline of 20 to 40 mm. Hg during the early part of the experiment. At this lower level it is often maintained for periods of two to three hours. There is shown, however, in some instances a slight upward trend of the curve beginning at the end of about the third hour after the administration of the insulin.

The records of pressure pulse obtained in an experiment of the type described above show very slight changes in the important parts of the curves. This is well illustrated in the tracings from a typical experiment shown in figure 1. In the segment marked *0-2* of this figure, that part designated *b-c* presents a sinusoidal form. A normal state of peripheral resistance gives pressure pulse tracings of the form portrayed in this record. On the other hand, previous studies have shown (16) that, when peripheral tonus is diminishing, this part of the pulse record takes on at first a skew curve contour, and finally, with extreme peripheral dilatation, it is little more than a continuous abrupt descent of the primary vibration. With these features in mind, it will be seen from segments *0-9* and *0-16* of figure 1 that the changes, in a result of this type, are not indicative of a primary reduction in the peripheral resistance factor of the circulation.

An analysis of the tracings relative to the heart frequency reveals an increase during the progress of the experiments in practically every instance. As a matter of convenience we have derived the curves of heart

rate from measurements of the heart cycle time, as shown by the records of pressure pulse or by the electrocardiogram. In order to give the curves the usual directional trend we have taken for the abscissa values the reciprocal of the cycle time. In figure 1 it will be seen, by comparing the curves *B* and *C*, that the change in heart rate does not occur until after the decline of blood pressure has progressed for some time. This result, however, is not shown in all instances. Some experiments show the

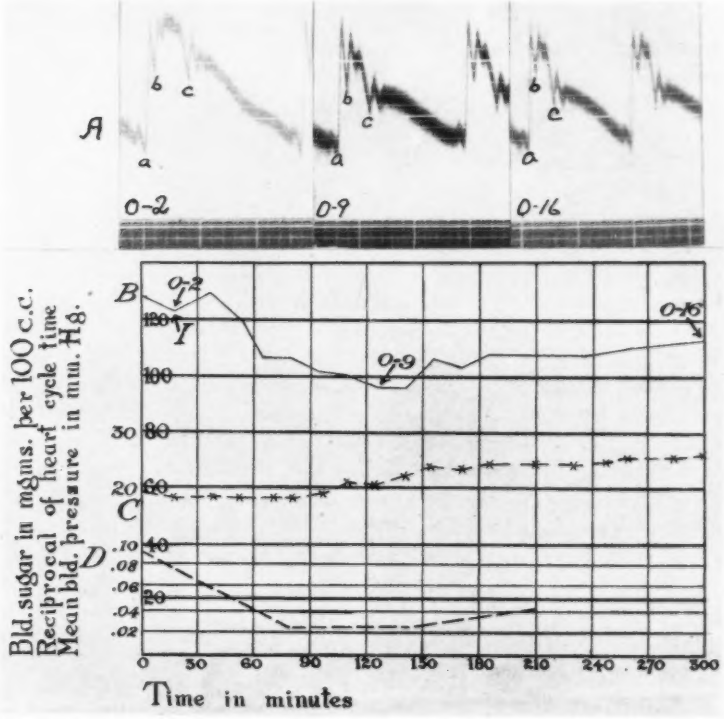

Fig. 1. *A*, carotid pressure pulse; *B*, mean blood pressure; *C*, heart rate from the reciprocal of the cycle length; and *D*, blood sugar concentration. *I* indicates the injection of 25 units of insulin per kilo body weight.

increase in heart rate coincident with the decline of blood pressure, while in other instances the initial increase in frequency precedes the beginning of the pressure change.

The significant feature of these results is that the change in heart rate appears not to be primarily conditioned by a change in blood pressure. Considered in this light, the increase in heart frequency noted in the previous experiments reveals information concerning the state of

cardio-dynamics. In line with recognized physiological principles, a heart rate increase may be expected to follow *a*, a decrease in peripheral resistance; or *b*, a decrease in the dynamics of the heart. Since we have not found evidence of a reduction in the peripheral resistance factor of the circulation, we interpret this change in the heart rate, which is apparently not primarily related to a fall in blood pressure, as a compensatory measure for a reduced cardiac contracting force.

The results may now be considered of that group previously designated as the second type of blood pressure response. The curves in experiments of this kind exhibit a gradual decline of pressure in the early part and then a rapid fall to a low level. The latter change usually begins at about the end of the second hour in the progress of the experiment and initiates in some instances a sudden fatal collapse, while in other instances a condition develops simulating circulatory failure. The records of pressure pulse show a normal contour throughout the phase of gradually reducing mean blood pressure. Such tracings have been obtained also in the early part of the phase of rapidly falling blood pressure. There is finally shown, however, in all of the experiments of this group a pressure pulse form characteristic of low peripheral resistance.

In three instances out of the eighteen experiments in which insulin was used in large doses, we have observed the development of a pulsus alternans. The results of one experiment showing this condition are here presented in figure 2. The mean blood pressure record shows during the first two and one-half hours of the experiment the usual gradual decline and the heart rate some increase during the corresponding period. There is shown also in the pressure pulse tracing for the same period little indication of a change in the peripheral resistance. At the point marked *P* on the blood pressure curve the pulse records exhibit a great reduction in the amplitude of every alternate beat. This condition progresses rapidly, and in the tracing shown in segment *0-11* it will be observed that the alternate contractions of the heart were too feeble effectively to open the aortic valves.

This hypodynamic state of the heart action soon led to a marked reduction in blood pressure. The pressure pulse tracings also took on in a short time a form indicative of a decreased peripheral resistance. There was apparently established a new cardio-vascular balance at a blood pressure level of about 70 to 80 mm. Hg, but the alternating character of the pulse was not changed. At the point indicated, *G*, on the curve an injection of glucose was given. This resulted in a complete restoration of the dynamic rhythm of the heart, as shown in segment *0-12*, figure 2. The damage previously done to the vascular system, however, was apparently too great, so while a restored rhythm had some beneficial influence, it was not sufficient to effect recovery.

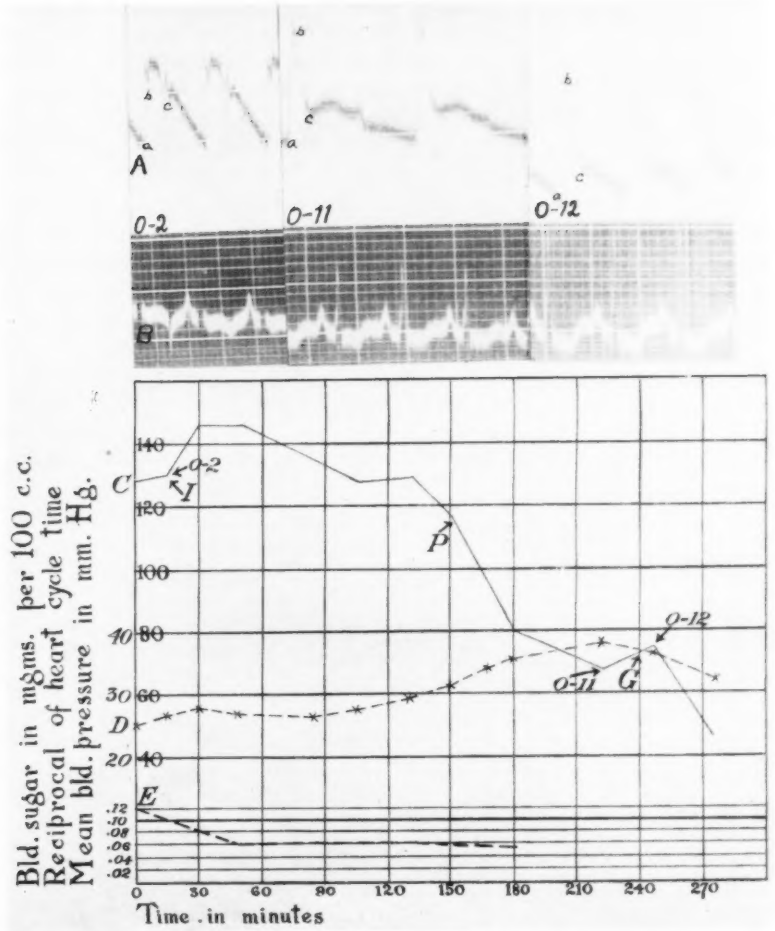

Fig. 2. *A*, carotid pressure pulse; *B*, mean blood pressure; *C*, the heart rate from the reciprocal of the cycle length; and *D*, the blood sugar concentration. *I* indicates the injection of 30 units of insulin per kilo body weight. *P* indicates the point of beginning of pulsus alternans. *G* indicates the injection of 0.20 gram glucose per kilo body weight.

Effects on the heart action. In this part of the experimental work studies were made upon the contractility and conductivity functions of the heart during periods of low blood sugar. The contractile response of the heart was followed by records of the intraventricular pressure, of the ventricular volume changes, and of the heart sounds. It should be noted in passing that the first two procedures have some disturbing effects on heart function. In evaluating the degree of alteration that results from the introduction of another factor, such as the effect of insulin, allowance has been made for the instrumental disturbances inherent in the methods used.

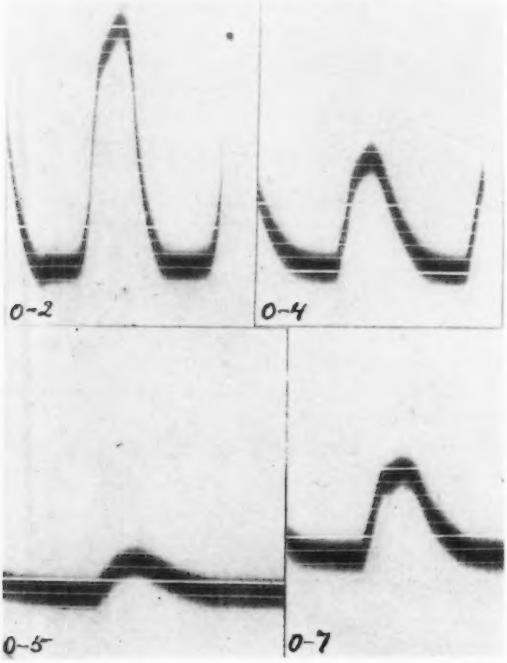

Fig. 3. Records of the left intraventricular pressure showing the decrease in contracting force, *0–2*, *0–4* and *0–5*, and the restoring effect of glucose, *0–7*.

The experimental results on intraventricular pressure show that the contracting power of the heart rapidly diminishes under the influence of insulin. Usually, in from sixty to ninety minutes the heart contractions become too feeble to maintain an adequate circulation and a fatal decline in blood pressure occurs. Figure 3 illustrates very well the course taken by an experiment of this kind. The segments *0-2*, *-4* and *-5* show

the progressive weakening of the force of the contractions, with the latter curve signifying mere pulsations of the organ. The rhythm is somewhat slowed, but always regular. Immediately following curve *0-5* in the course of this experiment an injection of glucose was given with the beneficial effects portrayed in the last curve, *0-7*, of this figure.

Time measurements taken from a typical experiment on intraventricular pressure reveal a lengthening of the isometric period from 0.039 to 0.041 second and a shortening of the total period of increasing pressure from 0.11 to 0.085 second. This change in the character of the contractile process developed in a period of one hour and was accompanied by a fall in blood sugar from 0.134 to 0.052 mgm. per 100 cc. of blood and a decline in blood pressure from 100 to 70 mm. Hg. The blood sugar concentration continued to fall and, at the end, ninety minutes from the beginning of the experiment, it was 0.012 per cent, with the heart exhibiting at this time a slow rhythm and weak contractions. These time changes in the contractile response of the ventricle indicate, we believe, that after insulin the tension developing capacity of the heart is slowed. On the other hand, the total phase of increasing pressure within the heart, which includes the first part of the ejection period, is diminished through a cutting down of the systolic ejection time.

An objection might be raised to the foregoing results on the ground that the drop in blood pressure produced immediate changes in the heart sufficient to account for the alterations in the contractile response. There can be little doubt that this factor plays some part. But the results with the cardiometer and phonocardiogram methods are significant in this connection. In three experiments in which the former method was employed there is shown an average shortening in the ejection period of 0.03 second during the first sixty minutes of the experimental period and an average fall in blood pressure of only 14 mm. Hg in the same interval. The phonocardiogram gives the systolic time from the interval between the first and second sounds. Since the taking of heart sounds requires no operative measures, this method completely rules out possible traumatic influences. In a typical experiment employing this method the results show that after a period of three hours and twenty-five minutes from the beginning of the experiment there had occurred a decline in blood pressure of 16 mm. Hg and a decrease in the systolic time of 0.03 second. The accompanying fall in blood sugar was from 0.14 to 0.022 mgm. per 100 cc. of blood.

A feature of interest in those experiments of this group in which complete circulatory failure took place was the absence of terminating fibrillation of the heart. It has been our observation in other work on the heart that the use of instruments directly upon this organ results eventually in fibrillation in a majority of instances. But we have not observed this

phenomenon develop in a single instance in animals with hypoglycemia from insulin, although the instruments were applied directly to such hearts according to the procedure usually employed. The outstanding type of failure has appeared more like a progressive weakening of the heart than of an acute malfunctioning of the organ.

The electrocardiogram shows no significant changes in the P-R interval or in the Q. R. S. group. It follows from this that the conduction function of the heart is not influenced by the hypoglycemia. In the T-wave, however, definite changes have appeared frequently in the course of these experiments. It is not possible to recognize a predominating type, but in general the alteration comes under one of the following categories: *a*, a change from a normal positive wave at the beginning to a very small, or even no T-wave at all, toward the end of the experiment; *b*, a change from a normal wave to one having a positive and negative component; and *c*, a change from a normal to a markedly negative one. Another instance of an unusual modification of this wave is shown in one experiment in which, beginning with a normal type, there follows in the successive records a gradual transition to a negative form, and finally a return to practically its original form toward the end of the experiment. These alterations in the T-wave do not appear to be due to an effect of the hypnotic as they were not present in the controls.

Effect of insulin on heart glycogen. The observations of Cruickshank (17) on the glycogen content of the heart in dogs not previously kept on a special diet show a variation from 0.42 to 0.631 per cent for the whole heart. The interesting fact of a close relation between the glycogen content of the heart and the store of this substance in the liver was early pointed out by Kulz (18). Selecting two dogs of widely different weight and subjecting each to severe work, after which they were sacrificed, this observer demonstrated in the larger animal a glycogen content of 0.62 per cent for the liver and 0.16 per cent for the heart, and in the smaller animal a glycogen content of 0.16 per cent for the liver and 0.05 per cent for the heart. These data suggest that, with a depletion of the glycogen stores of the body, the activity of the heart reduces the glycogen content of this organ to a very low level.

In our experiments evidence is given to indicate an alteration in the functional activity of the heart during insulin hypoglycemia. The glycogen content of the ventricular muscle was determined in three of these experiments with results as follows:

BODY WEIGHT	BLOOD SUGAR		HEART GLYCOGEN
	Beginning	End	
kgm.	*per cent*	*per cent*	*per cent*
6.	0.11	0.04	0.166
6.8	0.14	0.02	0.072
5.5	0.134	0.012	0.050

These observations are insufficient to warrant definite conclusions. They are presented here simply to record the fact that a low glycogen content is shown in hearts which previously had exhibited evidences of hypodynamic function.

Discussion. The results reported in the present paper show definite indications of circulatory insufficiency during insulin hypoglycemia. The outstanding features of the change in cardiovascular balance are the decrease in mean blood pressure and the increase in heart rate. Since the magnitude of blood pressure represents the resultant of the coördination of the factors, heart frequency, peripheral resistance and cardiac output, these results alone do not reveal the meaning of the change in heart rate. The observations on the pressure pulse records, therefore, are especially significant in showing little indication of a primary change in peripheral resistance. It follows from these considerations that the heart rate-blood pressure relation noted under the insulin effect, indicates some change in heart action whereby the output becomes diminished. Whether this change is due entirely to an alteration in its mechanism of energy conversion or is due in part to a change in the total blood volume, is not evident from the data at hand.

The experimental results on the heart directly leave little room for doubt that there are immediate changes in the contractile behavior of this organ. The main results in support of this view are the rapid decline in heart action to a feeble, slow rhythm without evidences of terminal fibrillation, and the decrease in systolic time. Moreover, in hearts exhibiting these features there was shown a remarkable augmenting action from injections of glucose solution. The beneficial effects of such injections may have been through *a*, the added fluid volume to the blood which facilitated the coronary circulation, or *b*, the giving to the heart a new supply of combustible material. The fact that only 30 to 60 cc. of fluid were added would seem to indicate that this may not have played a significant part in bringing about the result.

So far we have considered the direct evidence of a change in the functional activity of the heart in hypoglycemia. We have now to turn to certain observations that suggest in an indirect way a similar type of influence. A result of this kind is shown in the pulsus alternans which has appeared during the course of some of these experiments. The exact

factors concerned in initiating this peculiar cardiac behavior are not fully evident, but the conditions usually attending its appearance are such as clearly to suggest a change in the metabolic state of the heart. The fact that pulsus alternans developed in our experiments under the condition of hypoglycemia and was made to disappear by injections of glucose, gives additional evidence, we believe, that there is a definite change in the contractility function of the heart associated with the condition of maintained low blood sugar.

Additional evidence that changes of an unusual character take place in the heart mechanism under the influence of insulin is presented by the alterations observed in the T-wave of the electrocardiogram. Some differences of opinion prevail as to the full significance of the T-wave, but most writers are agreed that it is an expression of muscular potential relations. An alteration of this wave, therefore, signifies in general the influence of factors on muscular contractile behavior. The modification of the T-wave observed in these experiments is, in the light of this interpretation, an expression of some sort of a change in the muscular response of the heart.

The mechanism by which insulin in large doses influences the action of the heart is not clear. Additional experimental work is required to ascertain whether it is through some process that lessens the supply of combustible material that is in an available form or whether it is through a sort of inactivation or interference with the utilization of other forms of stored potential of the heart.

The interesting observations of Hepburn and Latchford (19) showing that, with the isolated heart, insulin has the effect of increasing its power of utilizing glucose suggested to us that a possible early effect of insulin might be an improved heart action until the store of blood sugar was materially decreased. We have some experimental evidence indicative of such an action, but the data do not warrant definite conclusions.

SUMMARY

1. The hypnotic iso-amyl-ethyl-barbituric acid used in these experiments proved well adapted to the purpose. It is apparently devoid of effect upon blood sugar concentration and has no disturbing influences on the circulatory system when used in doses of 50 to 60 mgm. per kilo body weight even during periods as long as three hours.

2. By employing large doses of insulin (25 to 35 units per kilogram) the blood sugar concentration was reduced to levels ranging from 0.012 to 0.05.

3. Mean blood pressure shows a moderate decline during the first two hours following the injection of insulin. There follows in some instances a partial recovery and in other instances a rapid decline. The pressure

pulse records indicate no primary changes in peripheral resistance. The heart rate is increased.

4. Intraventricular pressure records indicate a rapid decrease in the dynamic capacity of the heart. Such curves, when analyzed in conjunction with the records of heart volume changes and of heart sounds, indicate a slight lengthening of the isometric phase and a shortening of the total period of rising pressure and of systolic ejection time.

5. The phenomenon of pulsus alternans has appeared in some experiments so marked that the alternate beats completely failed to open the aortic valves.

6. There are no conduction changes in the heart evident from the electrocardiogram. The T-wave exhibits during the course of single experiments such changes as reduction in size, a splitting into a positive and negative component, and complete inversion.

7. The circulatory changes in insulin hypoglycemia present characteristics of a diminished functional capacity of the heart. The changes may be moderate, as shown by a slight fall in mean blood pressure with an accompanying increase in heart rate and little alteration in peripheral resistance, or it may present the essential features of circulatory failure shock.

Intravenous injections of glucose produced a remarkable improving effect on such hypodynamic hearts.

Acknowledgment is made to Eli Lilly & Company for generously supplying the iso-amyl-ethyl-barbituric acid and the insulin used in all of our experimental work.

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THE COLLOID CHEMISTRY OF PROTOPLASM

IV. The Heat Coagulation of Protoplasm

L. V. HEILBRUNN

From the Department of Zoölogy, University of Michigan, Ann Arbor

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One of the most general properties of protoplasm is its inability to remain active or to live at temperatures only slightly above the usual temperature of the environment. This is common knowledge. Davenport (1) has recorded some of the temperatures which produce death in various groups of animals and plants. Unfortunately his list is far from complete and it tends to stress the higher rather than the lower death temperatures. Generally most forms of animal life die at temperatures between 30 and 45 degrees and plants at temperatures between 40 and 55 degrees Centigrade. In temperate latitudes most marine fishes and quite a few fresh water fishes die at temperatures around 25 degrees (2), (3). Many other animals have a similar low death point. Matisse (4) found that the cuttle-fish Sepia died at 28 degrees. Frenzel (5) found that several species of Crustacea die at about 27 degrees, and Brauer (6) found the death point for two Phyllopods to be 18 degrees and 19 degrees respectively. Many animals and plants commonly live at temperatures only a degree or two below their death point, and death frequently results from a slight rise in the temperature of the environment.

The first effect of the heat is to produce anesthesia, the animal fails to respond to stimulation and its various activities cease. Longer exposure to heat or a slight increase in temperature is followed by death.

Although all active protoplasm is acted upon by heat the nature of this effect is not clearly understood. Some authors believe that heat produces a coagulation of the same sort as the typical coagulation of proteins in the test tube. Brodie and Richardson (7) take this view in their study of the effects of heat on muscle, and the same stand is taken by Brodie and Halliburton (8) in their study of the action of heat on nerve. The point of view of these authors has been sharply criticised by Meigs (9), and he has shown their evidence to be both unreliable and inconclusive.

It may of course be true that some types of protoplasm are killed by the coagulation of the proteins they contain, but such an explanation can

scarcely hold for those cases in which death occurs at 20 or 30 degrees. The coagulation of pure proteins usually occurs at temperatures above 50 degrees, and the description of a protein which coagulates at 30 to 40 degrees (10) is rather doubtful. Even in the case of heat rigor at 40 degrees, such as occurs in frog muscle, Vernon (11) was forced to discard the view that it could be caused by protein coagulation.

Some authors have attempted other types of explanation. Vernon in his study of muscle suggests that it goes into a sort of tetanus at higher temperatures. Winterstein (12) believes that heat acts by producing asphyxiation. Mayer (13) thought that heat death was caused by an accumulation of acid. None of these authors has advanced very significant arguments in support of his theory, and no two of them agree.

Quite aside from theory it is certain that heat causes some profound change in the protoplasm. Does this change involve a change in the physical state of the protoplasm? Many years ago Kühne (14) observed the effects produced by heat on protoplasm and he described these effects as coagulations. The heat rigor of muscle also apparently involves a coagulation, but this change may be a result rather than a cause of the death or inactivity of the muscle. In the physical study of protoplasm morphological changes are at best uncertain indices of changes in colloidal condition. Some surer criterion is necessary. By measuring the viscosity of the protoplasm one can determine colloidal change with some degree of accuracy.

In studying the effect of heat on protoplasm it is important to know whether temperatures which cause death produce a coagulation of the protoplasm, and secondly, whether such a coagulation precedes or follows the death of the cell. This problem and other problems concerned with the heat coagulation of protoplasm were studied at Woods Hole during the summer of 1923. The centrifuge method was used in making viscosity tests. The details of this method have already been discussed (15), (16). The material used in the experiments was the protoplasm of the sea-urchin egg and of the Cumingia egg. When these eggs are heated it is easy to demonstrate a coagulation or gelation within them. In the heat-killed protoplasm the granules which fill the cell are unable to move under the influence of a strong centrifugal force.

Coagulation occurs first and death follows later. Indeed it is possible to obtain a light coagulation, and then by returning the egg to the cold, to induce a recovery. The following experiments illustrate this point. They also indicate that the heat has some effect on the fatty materials of the cell. When heated eggs are centrifuged and are compared with normal eggs given the same centrifugal treatment, the first noticeable effect of the heat is in the gray cap, which is the layer containing the fatty materials.

July 15. At 11:12 a.m., Arbacia eggs were placed in a test tube containing sea-water at a temperature of 32.3 degrees. The temperature was maintained constant by immersing the tube in a de Khotinsky constant temperature bath. At 11:22 a.m., eggs from the hot sea-water and control eggs at room temperature were centrifuged simultaneously, the high speed handle of the Bausch and Lomb centrifuge being turned 40 times in 40 seconds. In both heated and control eggs the hyaline zone showed through a third of the egg, although it was not as clear as in the heated eggs. In the latter, moreover, the gray cap differed in appearing more diffuse. Another lot of eggs from the hot sea-water was centrifuged at 11:27½ a.m., and these eggs were given the same treatment as those of the previous lot. But in this instance the hyaline zone was but faintly indicated. Five minutes later, at 11:32½ a.m., another lot of eggs was removed from the warm sea-water. This lot was divided into two portions. The first portion was tested with the centrifuge immediately, and the second portion was placed in sea-water at room temperature. A centrifugal test of the first portion was carried out as before, but in this instance no hyaline zone appeared and a coagulative change had evidently occurred. The eggs removed from the warmth at 11:32½ a.m. were tested at 12:13 p.m. in the usual manner. They showed a hyaline zone plainly through a third of the egg, thus indicating that their protoplasm had returned to its original fluidity. They still showed an effect of the heat on the fatty materials of the cell, for the gray cap was wider and more diffuse than in similarly centrifuged eggs which had never been exposed to the heat.

July 16. At 10:31 a.m., Arbacia eggs were placed in sea-water at a temperature of 32.9 degrees, and this temperature was maintained constant as before. Centrifuge tests were made as in the preceding experiment. A test at 10:36 a.m. showed a hyaline zone through one-third of the egg in the heated eggs. In some of these eggs the gray cap was lacking after centrifugal treatment. At 10:41 a.m., the eggs in hot sea-water were still uncoagulated and showed zones after centrifugal treatment, although in this instance too, the gray cap was sometimes absent. Eggs tested five minutes later, at 10:46 a.m., either lacked a hyaline zone, or showed it only very faintly. Thus coagulation began at about this time. Eggs were removed from the hot sea-water at the time this last test was made, and also five minutes later, at 10:51 a.m., and they were placed in separate dishes of sea-water at room temperature. Both lots of eggs returned to cool sea-water were tested at 11:24 a.m. The first lot of eggs, those exposed to the heat for 15 minutes, showed a hyaline zone running through at least a third of the egg, the gray cap was lacking. The second lot, exposed to the heat for 20 minutes, showed a hyaline

zone in some of the eggs but not in others; this zone never appeared very plainly.

These experiments show that temperatures as low as 32 or 33 degrees are effective in causing a coagulative change within the protoplasm of sea-urchin eggs. This coagulation precedes any other visible death changes, and for a time it is reversible. Similar results were obtained in experiments with the eggs of the clam Cumingia. Heat death in the protoplasm of marine eggs is doubtless due to a coagulation. It is of interest now to study the nature of this coagulative change and to relate it if possible to the coagulations which occur outside of the cell in lifeless materials.

Many investigators have studied the heat coagulation of egg albumin. This protein coagulates very rapidly at a temperature of about 76 degrees, at somewhat lower temperatures a longer time is required. Chick and Martin (17) measured the time of coagulation at various temperatures both for egg albumin and for hemoglobin. They found a high temperature coefficient of coagulation. In the case of hemoglobin the coagulation rate increased 1.3 times for each degree rise in temperature For 10 degree rises in temperature the temperature coefficient would be 13.8 for the hemoglobin and about 635 for the albumin. Buglia (18) measured the time of coagulation of serum albumin at various temperatures and plotted a curve to illustrate his results.

In order to compare the heat coagulation of protoplasm with the heat coagulation of the proteins studied by Chick and Martin and by Buglia, the rate of coagulation of protoplasm at various temperatures was determined. Of course the problem is not so simple in the case of living cells. Protoplasmic coagulation involves no visible change and it is necessary to test the protoplasm from time to time to see if coagulation has occurred. These tests require time and the interval between tests introduces a source of error. Moreover protoplasm even of a given type is not always uniform.

The curves shown in the figure are almost self-explanatory. They show the approximate length of time necessary to produce coagulation at various temperatures. Constant temperature was maintained with the help of a de Khotinsky constant temperature apparatus. Of the two curves, the upper curve, or the one to the right, shows the conditions in the egg of Cumingia, the other curve is for the sea-urchin egg. In view of the fact that various lots of eggs from a number of different females were used in the experiments, it is surprising that such smooth curves could be obtained. However, one lot of Cumingia eggs proved exceptional. Two records were obtained from this lot of eggs and in both the time of coagulation was decidedly lower than was anticipated. In Cu-

mingia it is not uncommon to find eggs which instead of having the usual pink color appear white to the naked eye. The aberrant eggs were of this white variety, but it is not known whether this fact has any significance or not. The two measurements on this white lot of eggs are marked in the figure with squares rather than circles to distinguish them from the other measurements.

In determining the time of coagulation the viscosity method was used as a criterion of coagulation. The Cumingia protoplasm was considered gelled or coagulated when five turns of the high-speed centrifuge handle in five seconds failed to show any zones within the egg. This represents at least a threefold increase of viscosity. In the sea-urchin egg, the centrifugal treatment used as a test of coagulation was forty turns of the high-speed handle in forty seconds. Eggs which failed to show a hyaline zone after this treatment were said to be coagulated. Of course in both cases the criteria chosen were more or less arbitrary ones. Moreover they do not represent the same degree of viscosity increase, for the forty turns used in the Arbacia test would reveal even a twofold increase in viscosity, whereas the Cumingia test shows only a threefold increase.

Inspection of the curve shows that it has the same general form as the curve obtained by Buglia (18) in his study of the time of coagulation of serum albumin at different temperatures. Moreover the temperature coefficient of coagulation for protoplasm is also similar to that of the proteins. Chick and Martin (17) found that the temperature coefficient of rate of coagulation per degree rise of temperature is 1.3 for hemoglobin and 1.91 for egg albumin. From the curve it can be seen that the temperature coefficient of heat coagulation of protoplasm is somewhere between these two numbers.

There is some evidence therefore that the heat coagulation of protoplasm is essentially a protein coagulation. But there are objections to this interpretation. In the first place it is rather doubtful if proteins coagulate as a result of exposure to temperatures of 30 to 35 degrees. It is true that some workers have described heat coagulation of proteins at such low temperatures (10), (19), but these observations were made on extracts of tissues and the coagulations observed are apparently due more to the chemicals used in making the extraction than to any rise in temperature. In pure preparations of proteins, heat coagulation occurs typically at temperatures above 50 degrees.

Secondly, and this is more important, the heat coagulation of protoplasm is for a time reversible. Definite proof of such reversibility is furnished by the experiments cited previously. There is also some older evidence in support of this contention. Sachs (20) described a cessation of protoplasmic streaming in the heat-treated protoplasm of plants. This he called a "vorübergehende Wärmestarre des Protoplasma," and he found

it to be reversible if the heat treatment was not too prolonged. Gotschlich (21) found that the heat rigor of frog muscle could also be reversed in its early stages. In general, then, the heat coagulation of protoplasm is reversible and the heat coagulation of proteins is irreversible. This is an essential difference.

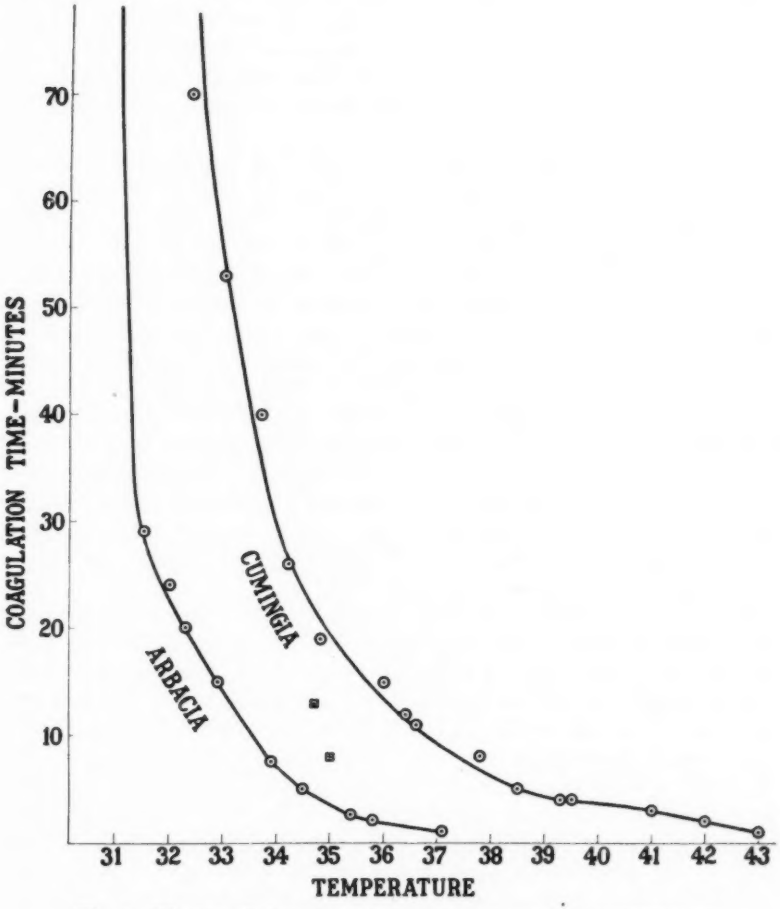

Fig. 1. The coagulation time of protoplasm at various temperatures

It thus appears that the heat coagulation of protoplasm is a process which can not be identified with the coagulation of proteins. Protoplasm is not pure protein and it certainly differs from protein in its essential behavior. The effect of heat in causing a destruction of protoplasm

is a widespread phenomenon and it is important to attempt to discover any facts which may furnish a clue as to what actually happens.

If the effect of heat is not on the proteins there must be other materials in the cell which are affected. It is possible that the heat coagulation of protoplasm is due primarily to an action of the heat on the fats and lipoids emulsified in the living substance. In the first paper of this series, it was pointed out that all protoplasm contains fatty substances in a state of emulsification. These emulsified or suspended fats and lipoids are an ever-present part of protoplasm, and they are probably as important as any other constituent in giving to protoplasm the qualities which make it alive.

The temperatures which cause death and heat coagulation of protoplasm, although they apparently could have no marked effect on the nature and physical state of the proteins of living substance, are the very temperatures which might be thought a priori to have a profound effect on the consistency and the physical state of the emulsified fats, for they are the temperatures which produce a liquefaction of the fats and lipoids which have been extracted from living material. Furthermore the liquefaction, or at any rate the solution, of the fats of protoplasm generally results in a coagulation. Many fat solvents cause a coagulation of the protoplasm of sea-urchin egg (22), and the same effect has been noted in the case of ether acting on the protoplasm of plants (23). The action of ether and of heat is quite comparable, for dilute ether solutions cause increased fluidity of protoplasm, and slightly higher concentrations of ether cause coagulation (22), (24). Similarly a rise in temperature causes an increase in the fluidity of protoplasm, while a further rise in temperature causes coagulation (25).

It is not at all unlikely therefore that the effect of heat on protoplasm is primarily an effect on the fatty substances. In favor of this view there are two more direct lines of evidence. In the first place the heat which causes coagulation can be seen to produce a visible effect on the fatty materials of the sea-urchin egg cell. When heat treated cells are centrifuged before coagulation has taken place, the fat which settles out at the lighter pole of the cell is seen to be modified. Instead of being compressed into a very small cap at one pole of the cell, as in normal eggs, the fat in the heat treated eggs is often found to be more diffuse. Probably the fat particles are smaller and for this reason offer a greater resistance to the centrifugal force.[1] Still longer heat treatment prior to coagulation may cause a complete disappearance of the fatty zone, and this may be taken as an indication that the fat has been completely dissolved.

[1] The theoretical reason for this effect of size is given in an earlier paper. See Heilbrunn, Journ. Exper. Zoöl., 1921, xxxiv, 417.

Another indication that the action of heat involves an alteration of the fatty materials of the cell is the fact that the coagulating effect of heat is hastened by the presence of a small concentration of ether. Apparently anything that hastens the solution of the fats and lipoids favors the heat coagulation of the protoplasm.

July 20. Sea-urchin eggs were divided into three lots. Lot A was placed in 1 per cent ether in sea-water at a temperature of 32.0 degrees, lot B in pure sea-water at the same temperature, lot C was placed in 1 per cent ether in sea-water at room temperature (about 25 degrees). After 12 minutes' exposure to the warm temperature the etherized eggs when centrifuged at the rate of 40 turns of the high speed handle in 40 seconds showed only indications of zones, after 16 minutes when centrifuged at the same rate and for the same time they showed no zones at all, whereas the unetherized eggs in lot B with the same centrifugal treatment showed zones clearly, the hyaline zone extending through a third of the egg. The eggs in lot B began to be coagulated only after 24 minutes. The eggs in lot C (1 per cent ether at room temperature) showed no coagulation when tested after exposure of 68 minutes.

July 21. Sea-urchin eggs were placed in 1½ per cent ether in sea-water. After 15 minutes in this solution they were taken out and placed in pure sea-water at a temperature of 32.2 degrees. Within 12 minutes they were all coagulated, although the coagulation time at this temperature for other eggs of the same lot was found to be 24 minutes.

These experiments show that small concentrations of ether hasten heat coagulation in the sea-urchin egg. Similar results were also obtained for the egg of Cumingia. Since, therefore, the temperatures which produce heat coagulation are seen to have a visible effect on the fatty substances in protoplasm, and since moreover, the fat solvent ether hastens such coagulation, it is fair to assume that the heat coagulation of protoplasm may be related in some manner to a change in the physical state of the fatty substances of the cell.

If this is true it might be thought that animals which suffer heat death at relatively low temperatures would have in their cells fats of lower melting point. Of course no absolute correspondence could be expected, for the fats most readily extracted from animals are not the fats of the protoplasm. Often large masses of fat are stored in adipose tissue, and in this form the fat is not really a part of the protoplasm. The blubber of the whale is the best example of fat stored in this manner. Nevertheless it is perhaps worth while to point out that most of the fatty materials which can be extracted from fishes are fluid at room temperatures, whereas those obtained from ordinary mammals are commonly solid, and

this is correlated with the fact that fishes die at much lower temperatures than mammals. It is probable also that plants of temperate climates have fats of lower melting point than those of the tropics; this is true at any rate for the seeds (26). In this case too there is a correlation between lower death temperature and lower melting point of fat. Such a correlation by no means constitutes a proof that heat death involves some change in the fats, but it is at least evidence worth considering. Much stronger evidence has already been presented.

SUMMARY

1. The heat death of sea-urchin and Cumingia eggs is preceded by a coagulative change in the protoplasm.

2. This coagulation occurs much more rapidly as the temperature is raised. The variation of time of coagulation with temperature has been recorded. The temperature coefficient of the process is of the same order of magnitude as that which is found in the heat coagulation of proteins.

3. Heat coagulation of protoplasm differs from heat coagulation of proteins in being reversible.

4. It is apparently associated with some change in state of the fatty materials of the cell. Such a change is indicated by visible differences in the fats of heat treated protoplasm and also by the fact that small concentrations of ether hasten the heat coagulation of protoplasm.

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A SIMPLE METHOD OF MEASURING THE INTENSITY OF ULTRAVIOLET LIGHT WITH COMPARATIVE RESULTS ON A NUMBER OF PHYSIOLOGICAL REACTIONS

JANET H. CLARK

From the School of Hygiene and Public Health of the Johns Hopkins University, Baltimore, Maryland

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PART I. THE LITHOPONE UNIT OF ULTRAVIOLET INTENSITY. During the last few years a great impetus has been given to the therapeutic use of ultraviolet light by the discovery of its curative action in rickets. Up to the present time, however, the doses given in different hospitals are only specified by the nature of the light source, the distance of the patient and the time of exposure. If a standard iron arc were used throughout, the specifications would be sufficient to make comparisons possible between the doses. However, the quartz mercury arc, which is easier to operate and usually more efficient than the iron arc, is generally used. Mercury arcs of different makes vary greatly in intensity and they all deteriorate with use so that, even when the current and voltage are specified, one lamp may differ very much from another in intensity.

In quantitative experimental work with ultraviolet light the energy in each line of the ultraviolet spectrum of the source should be measured by means of a thermopile or some other heat-measuring instrument. Such accuracy as this is both unnecessary and impracticable in most clinical work, and also in laboratory work of a qualitative nature. There is, therefore, a definite need for some method of determining ultraviolet dosage which is simple enough for general clinical use and at the same time fairly accurate. The darkening of a photographic plate has been used but, as the photographic plate is sensitive to wave lengths longer than those which produce physiological effects, it is not very satisfactory. Many photochemical reactions have been used to measure the intensity of ultraviolet light but have not been tested to any extent for their accuracy in measuring the rate of physiological light reactions. It is believed that the darkening of lithoponé paint in ultraviolet light, which is described in detail in this article, is simple enough, and at the same time accurate enough, to be of considerable practical value, especially in clinical work. Lithopone paint is manufactured both in light sensitive and light resistant

form and the following tests were made with a sample of Green Seal Lithopone from the Du Pont Company.

Dr. A. H. Pfund, in a paper presented at the Twenty-sixth Annual Meeting of the American Society for Testing Materials in June, 1923, made a careful study of the sensitivity of lithopone to ultraviolet light, and it was at his suggestion that the rate of darkening of lithopone was compared with the rate of various physiological light reactions. Lithopone, in the form of a powder, was made into a water paste and pressed between a quartz plate and a glass one. The quartz side was then exposed to different wavelengths in an ultraviolet spectrum by means of a quartz monochromator, the energy in each wavelength having been first adjusted to a standard value by means of a vacuum theromopile. In this way Pfund found that lithopone is practically insensitive to wavelengths longer than 320mμ and from 300 to 230 mμ the sensitivity is practically uniform. It shows no perceptible darkening when radiated through ordinary window glass which cuts out wavelengths shorter than 320 mμ. Since the sensitivity of lithopone to light begins at approximately the same wavelength where physiological light reactions begin, and since the sensitivity is practically constant throughout the ultraviolet, it seemed well adapted to the measurement of ultraviolet intensities and was accordingly tested.

Method: A spatula full of lithopone powder is placed on a glass plate and enough distilled water added to make a moderately thick paste. A piece of transparent quartz is pressed down on the lithopone and the quartz and glass plates are held together by elastic bands, thus insuring a thin layer of lithopone which should be uniform and free from air bubbles. In this way evaporation and drying, which affect the sensitivity of the lithopone water paste, are prevented. The sample is then placed in a holder with the quartz side out and exposed normally to a beam of ultraviolet light from the light source under investigation.

Samples of lithopone, prepared in this manner, were exposed for various lengths of time at a number of different distances from a standard iron arc. This arc is run on 110 D.C. at 6 amperes and 35 volts. A full description of this arc is given by Pfund (1). It consists of a lower electrode of $\frac{3}{8}$ inch iron rod with a $\frac{1}{4}$ inch hole bored to a depth of $\frac{3}{16}$ inch in the upper end for holding a bead of iron. The upper electrode, which is positive, consists of a piece of $\frac{1}{4}$ inch soft iron which projects about $\frac{1}{8}$ to $\frac{1}{4}$ of an inch from a cylindrical brass radiator.

Immediately after radiation the reflection factor of the exposed lithopone was measured by means of a Macbeth Illuminometer, by comparison with a test plate of know reflection factor. The reflection factor of the lithopone under quartz, before exposure, was 82 to 83 per cent. The reflection factor is diminished by the super-imposed quartz plate for a

smooth lithopone paste in glycerine reflects 90 per cent. The results obtained in this way were very consistent and easily repeated and the curves plotted from the results are shown in figure 1. The curves are nearly linear until a reflection factor of 50 per cent is reached, after which the rate of darkening decreases rapidly.

The lithopone unit of ultraviolet intensity. Since the time of first darkening is too short to be a useful ultraviolet unit it is proposed that the intensity required to darken lithopone to a reflection factor of 50 per cent be called the lithopone unit of ultraviolet intensity. (This means an

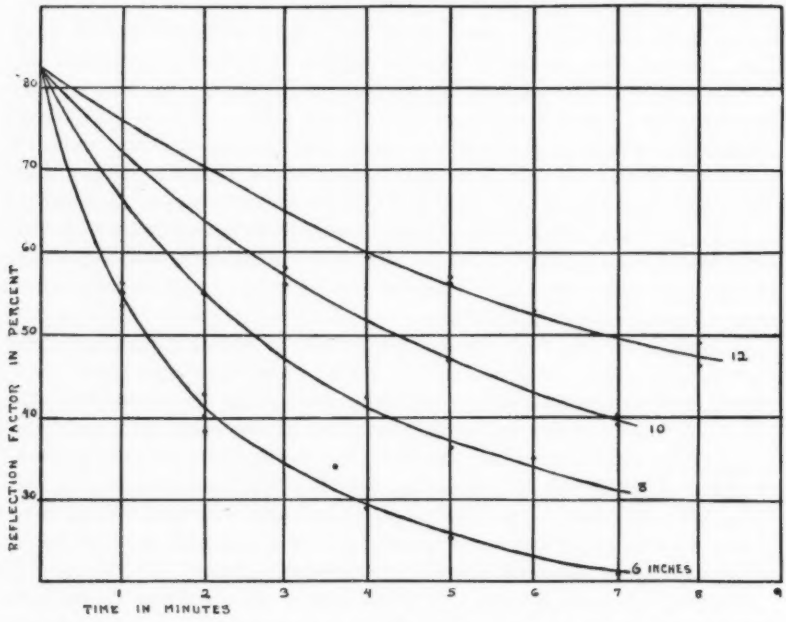


Fig. 1. Curves showing rate of darkening of lithopone at different distances from the standard iron arc.

actual reflection factor of 50 per cent and not 50 per cent of the original reflection factor of 82 per cent.) This unit may be easily determined in practise by exposing a sample of lithopone, prepared as described above, to the source of ultraviolet light, at the distance which is to be used in making an exposure. By comparing the darkened lithopone with a sample of grey paint having a reflection factor of 50 per cent, the time necessary to darken lithopone to the same reflection factor can be determined. Knowing the time required to give one lithopone unit of ultraviolet light at a certain distance from a given source, it is possible to

calculate roughly the time required to give a lithopone unit, at any other short distance from the same source, by the inverse square law.

In figure 2 (*A*) the time necessary to darken lithopone to a reflection factor of 50 per cent (i.e., the time required to give one lithopone unit of ultraviolet light) is plotted against the square of the distance from the source for the three arcs used in this investigation. Source I is a quartz

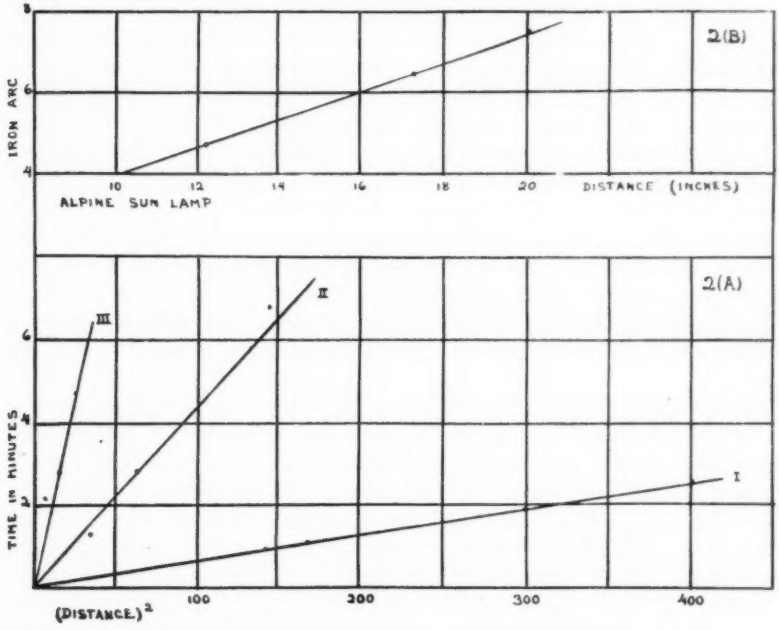


Fig. 2. (*A*) Time required to give one lithopone unit plotted against the distance squared for three sources of ultraviolet light.

I—Alpine Sun quartz mercury arc (110 D.C)

II—Standard iron arc

III—Cooper-Hewitt Lab-arc (110 A.C)

(*B*) Equivalent distance curves for the Alpine Sun lamp and the standard iron arc derived from (*A*)

Abscissae—Distance from Alpine Sun lamp

Ordinates—Distance from iron arc

mercury arc from the Hanovia Chemical Company known as the Alpine Sun burner. It was operated at 58 volts and 3 amperes on the 110 D.C. circuit. Source II is the standard iron arc described above, run on the 110 D.C. circuit at 35 volts and 6 amperes. Source III is a quartz mercury arc from the Cooper-Hewitt Company, known commercially as the Lab-arc, and operated on 110 A.C. The intensity of this arc is probably greater

when run on D.C. but it was run on A.C. for the sake of comparison with the other two arcs. Figure 2 (*A*) shows that the inverse square law holds very well for the rate of darkening of lithopone for the short distances used where the rate of darkening was fairly rapid. From this figure it is possible to calculate the time necessary to give a lithopone unit at other short distances from these three arcs.

In figure 2 (*B*) an equivalent distance curve is given for sources I and II, that is the distances from these sources which require the same length of time to give one lithopone unit. In other words, lithopone darkens at the same rate 6 inches from the standard iron arc (source II) and 16 inches from the quartz Alpine Sun lamp (source I); 7.5 inches from source II and 20 inches from source I and so on. If the lithopone unit is a feasible unit for the measurement of ultraviolet energy in physiology and medicine these equivalent distances, as determined by lithopone, should also be equivalent in producing such well-known physiological effects as the death of bacteria, the production of an erythema and so forth. In the second part of this paper the equivalent distance curves of the three sources determined by means of various physiological reactions, are compared with their equivalent distance curves as determined by the lithopone unit.

PART II. THE DEGREE OF ACCURACY WITH WHICH THE LITHOPONE UNIT MEASURES PHYSIOLOGICAL REACTIONS TO ULTRAVIOLET LIGHT. The mercury arc and the iron arc probably have entirely different energy curves in the ultraviolet, although thermopile measurements have not been made as yet in the ultraviolet spectrum of the iron arc. Pfund has shown that the sensitivity of lithopone is practically constant in the ultraviolet but measurements of physiological reactions, with wavelengths of equal energy, have shown that the sensitivity of bacteria increases as the wavelength decreases (2) while the sensitivity of the skin to erythema has a very decided maximum at wavelength 300 mμ (3). If therefore seemed extremely unlikely that the equivalent distance curves from lithopone would coincide with equivalent distance curves determined from physiological reactions. However, experiments with the three arcs show a remarkably good agreement for some reactions.

The Alpine Sun lamp was always run for five minutes before making an exposure and after that the intensity remained quite uniform. The iron arc remained very constant and there was no trouble in repeating results with it. The Lab-arc however was still increasing somewhat in intensity at the end of a ten minute run so that it was more difficult to obtain consistent results with it, even when it was run for ten minutes before any readings were made. The Alpine Sun lamp was, unfortunately, broken, when the erythema dose had been determined for one distance

only. Except for the erythema dose with the Alpine Sun lamp the results were repeated and are reasonably accurate. They are shown graphically in figure 3 (*A*, *B*, *C*, *D*). In figure 3 *A*, *B* and *D* the curves for source I (Alpine Sun lamp) were determined partly by points at greater distances which are not represented in this figure.

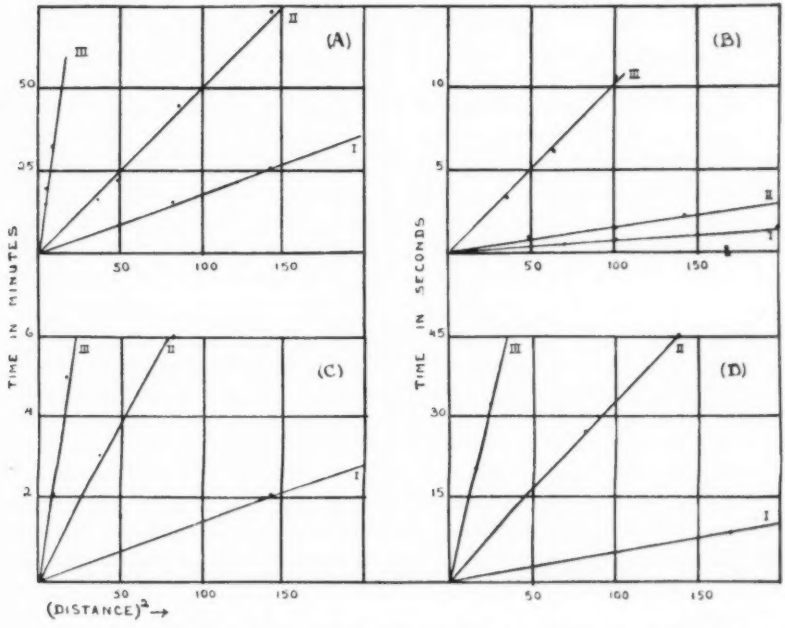


Fig. 3. (*A*) Time, in minutes, required to coagulate egg albumin plotted against the distance squared for all three sources.

(*B*) Time, in seconds, for discharge of electroscope plotted against the distance squared for all three sources.

(*C*) Time, in minutes, required to produce an erythema plotted against the distance squared for all three sources.

(*D*) Time, in seconds, required for lethal action on bacteria plotted against the distance squared for all three sources.

In figure 3 (*D*) the length of time necessary to kill bacteria is plotted against the distance squared for all three sources. An emulsion of a 24-hour culture of *B. coli* was spread on the surface of an agar plate and exposed normally to the beam of ultraviolet light with the cover of the petri dish removed. By means of a tin disc, from which a sector had been removed, different sectors of the same plate were given different exposure times and the lethal point established to within two seconds.

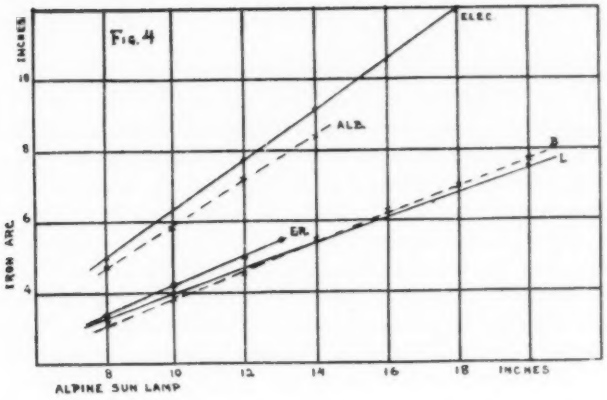
In figure 3 (*C*) the length of time necessary to produce a just perceptible erythema on the skin of the inside of the forearm is plotted against the distance squared for all three sources.

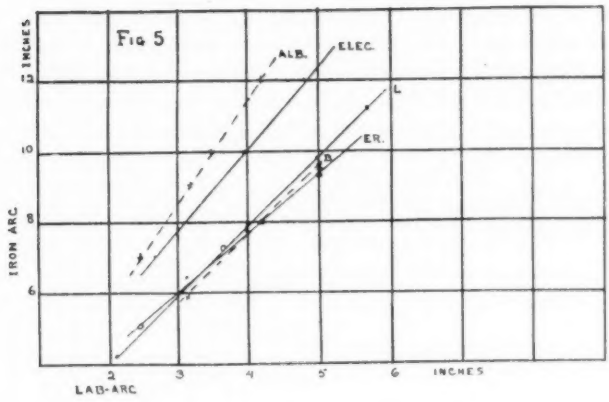
Figure 3 (*A*) shows the rate of coagulation of egg albumin at different distances from all three sources. The egg albumin was prepared as described in a previous paper by the author (4) and was exposed in small quartz test tubes. Before exposure the egg albumin was adjusted to pH 6.2 and the appearance of the first faint cloud was taken as the coagulation time.

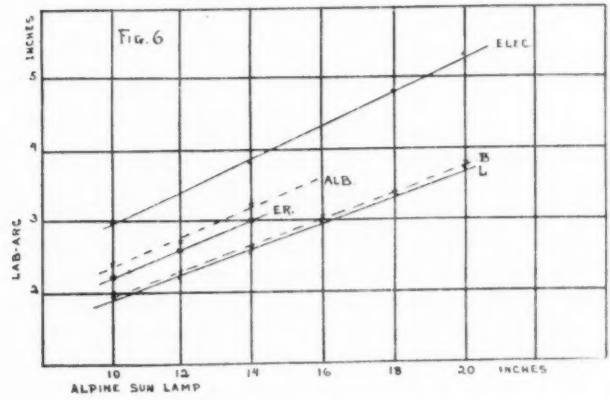
Figure 3 (*B*) shows the rate of discharge of an electroscope attached to a zinc plate 1.5 inches in diameter and negatively charged. This plate was polished with emery paper before each set of exposures. The time of discharge was the time it took for the gold leaf to fall between two fixed positions marked by cross hairs. This measures the rate of emission of photoelectrons from the zinc plate under ultraviolet light. Zinc starts to emit electrons at approximately wavelength 300 $m\mu$ and the rate of emission increases with decreasing wavelength. This is a purely physical phenomenon. The author has shown (4) that the coagulation of egg albumin is due to the emission of photoelectrons and, when no substances are present with which it can react chemically, this process is also a purely physical phenomenon. The darkening of lithopone, the killing of bacteria, and the production of an erythema are presumably photochemical reactions initiated by the emission of photoelectrons under light. It is not surprising therefore, as will be shown later, that the equivalent distance curves for the killing of bacteria, erythema, and the darkening of lithopone, are parallel while the equivalent distance curves for the coagulation of egg albumin are parallel to the electroscope curves but not to the ones from lithopone.

From the results plotted in figure 3 equivalent distance curves were derived and are given in figures 4, 5 and 6, together with the equivalent distance curves from lithopone. Figure 4 gives equivalent distances for the Alpine Sun lamp compared with the standard iron arc. Figure 5 gives equivalent distances for the Lab-arc and standard iron arc and figure 6 for the Lab-arc and Alpine Sun lamp.

The three figures (figs. 4, 5, 6) show that the lithopone unit gives an excellent measurement of the ultraviolet intensity of these three sources as far as the lethal action on bacteria and the erythema dose are concerned. When the standard iron arc is compared with either of the quartz mercury arcs the equivalent distance curve derived from the rate of discharge of the electroscope has a different inclination from the lithopone equivalent distance curve. The rate of coagulation of egg albumin, which is also a purely photoelectric phenomenon, is measured better by the rate of discharge of an electroscope than by the darkening of lithopone.







Figs. 4, 5, 6. Equivalent distance curves derived from *L*, lithopone, *B*, lethal action on bacteria, *Er*, erythema, *Elec.*, electroscope, *Alb.* coagulation of egg albumin.

Fig. 4. Alpine Sun lamp and iron arc.

Fig. 5. Lab-arc and iron arc.

Fig. 6. Alpine Sun lamp and Lab-arc.

When the two mercury arcs are compared all the equivalent distance curves are closely parallel and the rate of coagulation of egg albumin falls between the rate of darkening of lithopone and the rate of discharge of an electroscope.

From the very close agreement of the lithopone equivalent distance curves and the equivalent distance curves from bactericidal action and from erythema dose, it seems that the lithopone unit should be very useful in comparing the ultraviolet energy of different sources, determining the efficiency of new sources, and following the rate of depreciation of old sources.

This work was done with a small sample of light-sensitive lithopone and when the physiological tests showed that the lithopone unit might become of general practical use, a supply of lithopone was ordered which turned out to be slightly more light sensitive than the old sample. I have standardized this new lithopone for my own use and determined the number of lithopone units necessary to produce certain effects. (See table 1.)

TABLE 1

Number of lithopone units necessary to produce certain physiological effects (new lithopone)

	STANDARD IRON ARC	ALPINE SUN LAMP	LAB-ARC	AVERAGE NUMBER OF LITHOPONE UNITS
Lethal action on *B. coli*	0.15	0.15	0.16	0.153
Erythema dose	2.3	2.0	2.0	2.1
Coagulation of egg albumin (pH 6.2)	14.8	27.0	30.0	24.0

The time required to give one lithopone unit with the new lithopone is shorter than the time required to give the same darkening with the old lithopone in the ratio $\frac{1}{1.35}$. Therefore to produce the same action in various physiological effects 1.35 times as many lithopone units are required with the new lithopone as with the old. In table 1 are given the number of lithopone units necessary to produce an erythema, kill bacteria and coagulate egg albumin, determined in terms of the new lithopone. As would be expected from the equivalent distance curves, the number of units required to produce a certain effect are practically identical for all three sources for bactericidal action and erythema dose. The coagulation of egg albumin, which is very inaccurately measured by means of lithopone, varies with the different sources.

Therefore, with any given source of ultraviolet light, if the time of exposure is determined which will darken lithopone to a reflection factor of 50 per cent at a certain distance, then this time multiplied by 2.1 will

produce a just perceptible erythema, and multiplied by 0.153 will kill *B. Coli* at that same distance. It should be remembered, of course, that the number of lithopone units needed to produce an erythema probably varies with different individuals and also with different areas of skin on the same individual.

It should also be remembered that the inverse square law has only been shown to hold when the darkening is relatively rapid, that is, where it does not take over six minutes to darken lithopone to a reflection factor of 50 per cent. A few tests at distances, where it should have taken half an hour to darken lithopone to this extent, showed that at these distances the rate is somewhat slower than would be expected. It is very likely that the rate of erythema and bactericidal action would be slowed down in the same ratio but this has not been tested experimentally. In the absence of definite knowledge on this point it is best to use the lithopone unit only at distances where it takes exposures of six minutes or less to give one lithopone unit of ultraviolet light.

I shall be glad to give some of the new standardized lithopone to anyone interested in using this type of ultraviolet energy measurement and can also give them glass slides painted with a grey paint having a reflection factor of 50 per cent. If the unit should come into general use it is hoped that some firm will undertake to supply quartz plates painted on one half with a grey paint of reflection factor 50 per cent, so that lithopone under the other half can be exposed to a match.

SUMMARY

The intensity necessary to darken lithopone paint to a reflection factor of 50 per cent is proposed as a unit of intensity of ultraviolet light. This lithopone unit gives a reasonably accurate measurement of the efficiency of an ultraviolet light source in producing erythema and killing bacteria.

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